

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: FW: confirmation of animals to CO
Date: Tuesday, January 06, 2015 10:28:00 AM

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Wednesday, December 24, 2014 10:54 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Cc: Clarke, Patrick R. - APHIS
Subject: confirmation of animals to CO

By my count as of today.....and we have received almost all of the results from NVSL to help with the decision making.....You all will be taking 7 2014 calves, 4 seronegative cows and an unknown number....but presumably about 3..... cows that were injected with GonaCon in May 2014 but are pregnant.

Bring your big trailer..... ☺

From: [Rhyon, Jack C \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: FW: Consumption of Bison from GonaCon Experiment
Date: Tuesday, July 26, 2011 12:12:44 PM

FYI

From: Stephens, Stephanie H (APHIS)
Sent: Tuesday, July 26, 2011 12:07 PM
To: Rhyon, Jack C (APHIS)
Subject: RE: Consumption of Bison from GonaCon Experiment

Jack-I understand your thoughts on this. EPA's asking for this because they rarely allow a commodity from an unapproved pesticide to be consumed. Since GonaCon is not yet officially approved for use on bison, they don't want them to be consumed. The fact that it is approved for use on deer doesn't factor into this. I know that doesn't seem entirely rational, but it's a reality we have to face...

In any case, thanks for the confirmation. I'll pass this along to EPA, then we should be getting something in writing that says we don't have to do an EUP.

From: Rhyon, Jack C (APHIS)
Sent: Tuesday, July 26, 2011 11:24 AM
To: Stephens, Stephanie H (APHIS)
Cc: McCollum, Matthew P (APHIS); Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); Clarke, Patrick R. (APHIS)
Subject: RE: Consumption of Bison from GonaCon Experiment

Stephanie,

I find this a little crazy since the vaccine is approved by EPA for use in wild deer. Are they ever consumed? Anyway, if that is what they want, we will assure them that the vaccinates (not the controls or bulls or calves) will not be consumed.

Jack

From: Stephens, Stephanie H (APHIS)
Sent: Tuesday, July 26, 2011 9:05 AM
To: Rhyon, Jack C (APHIS)
Subject: Consumption of Bison from GonaCon Experiment

Hi Jack-

I've been having discussions with EPA and with Montana Department of Agriculture on our proposal to not obtain an Experimental Use Permit for the pesticide-related FIFRA requirements. EPA's generally in agreement that since the study is conducted in a confined area with fences, the EUP isn't necessary. The only remaining issue is that EPA wants us to guarantee that the bison from the experiment won't be consumed. Can we absolutely guarantee this?

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: Contraception permit sent to fax
Date: Friday, September 30, 2011 4:23:19 PM

I got this close. If you find it please let me have a copy.
Thanks,
Jack

-----Original Message-----

From: Christie_Hendrix@nps.gov [mailto:Christie_Hendrix@nps.gov]
Sent: Thursday, May 12, 2011 10:41 AM
To: Rhyan, Jack C (APHIS)
Cc: Rick_Wallen@nps.gov; PJ_White@nps.gov
Subject: Contraception permit sent to fax

Hi Jack,
The Superintendent has provisionally approved your request to obtain animals for the bison contraception study. We just sent the research and collection permit via fax, to 970-266-6138. If you could please return to us via fax today, it would be very helpful. We will then forward the document over to the superintendent for signature, and send you a final copy.
Thanks,
Christie Hendrix

Christie L. Hendrix
Research Permit Coordinator, Yellowstone National Park P.O. Box 168 Yellowstone NP, WY 82190
(307) 344-2234 office
(307) 344-2211 fax

Research Permit Page: <http://greateryellowstonescience.org/research/yell>
Greater Yellowstone Science: <http://greateryellowstonescience.org/>

*Please do not send files larger than 1.5 MB.

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: Draft Version of Bison Quarantine Protocol
Date: Thursday, January 05, 2012 11:48:27 AM

So who do we want to do the stat analysis for the BQFS...Barbra? Or someone at NWRC? Or maybe a fairly new PhD they call "Pauline"????Aha!!!

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: McCluskey, Brian J - APHIS
Sent: Thursday, January 05, 2012 8:28 AM
To: Clarke, Patrick R. - APHIS; Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: Re: Draft Version of Bison Quarantine Protocol

You are right, I felt we needed something ready if a real push came for "commercial" quarantine. I don't think we need to share this outside of this group until asked. In the meantime analysis is warranted.

Sorry for any confusion.

Brian

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 04, 2012 03:49 PM
To: McCluskey, Brian J - APHIS
Cc: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: RE: Draft Version of Bison Quarantine Protocol

Brian,

We just received the final test results on the 2nd cohort yesterday. We haven't started any formal analysis as yet.....we were under the impression you needed this sooner rather than later for the IBMP folks.....that even though the study was incomplete, that we could/should produce a best "guess" protocol based on our experience and the culture/test results in hand.

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: McCluskey, Brian J - APHIS

Sent: Wednesday, January 04, 2012 12:36 PM

To: Clarke, Patrick R. - APHIS

Cc: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyon, Jack C - APHIS; Nol, Pauline - APHIS

Subject: RE: Draft Version of Bison Quarantine Protocol

Ryan,

I wouldn't mind seeing the hard data as well. What kind of analysis was done to determine the overall time periods of quarantine for the various classes of bison? The study seems to lend itself well to a survival analysis.

Brian

From: Clarke, Patrick R. - APHIS

Sent: Wednesday, January 04, 2012 11:41 AM

To: McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Subject: Draft Version of Bison Quarantine Protocol

Brian, Don

The Bison Group (Jack, Matt, Pauline, Becky and I) put our heads together and came up with this draft. Even though the data from the BQFS has not been fully evaluated, this document is our recommendation for a quarantine protocol to be used for an Approved Bison Quarantine Facility. This protocol is based on our experience from the BQFS.

The original protocol (2003 UM & R), as put together by a variety of experts based primarily on their prior experience and knowledge of the disease in cattle, was essentially untried. We felt that the BQFS validated and proved this original protocol for bison, which is why what we have sent you does not radically depart from the original framework. The one significant change was removing the stipulation for post quarantine testing.

We passed this by John B., Arnie G., Mark C. and Don Evans. There were no major concerns except that Don actually wanted to see the data to get a feel for the numbers of animals/tests used in the study.

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: [Rhyon, Jack C \(APHIS\)](#)
To: [Frey, Rebecca K \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: FW: EA for Gonacon
Date: Tuesday, August 02, 2011 8:57:49 AM

FYI

From: Stephens, Stephanie H (APHIS)
Sent: Tuesday, August 02, 2011 7:35 AM
To: Rhyon, Jack C (APHIS)
Subject: RE: EA for Gonacon

Hi Jack- We were assuming an injection initiation date of 03/01/2012. So we are targeting announcement of the EA on 11/30/2011. The end of the 30-day comment period would be 12/30/2011, and that would give us time to then review/respond to comments and publish the FONSI by 02/28/2012.

If the comment period were extended beyond 11/30, it would put pressure on the completion of the process, but we have built a little flexibility into it in case that happens. Please let me know if we've made any incorrect assumptions about when the experiment will start.

Thanks,

Stephanie

From: Rhyon, Jack C (APHIS)
Sent: Monday, August 01, 2011 11:53 AM
To: Stephens, Stephanie H (APHIS)
Cc: Frey, Rebecca K (APHIS)
Subject: FW: EA for Gonacon

Stephanie,
Any approximation on the EA timeline?
Jack

From: Frey, Rebecca K (APHIS)
Sent: Monday, August 01, 2011 11:43 AM
To: Rhyon, Jack C (APHIS)
Subject: EA for Gonacon

HI,
We have to go to anohter IBMP meeting this week. Any timeline on the EA that we can announce....even if it is just a guess??

Thanks,
Becky

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: Final Report on 13-002818 For DR Jack C Rhyan
Date: Wednesday, June 19, 2013 10:52:18 AM
Attachments: [13-002818_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:17 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-002818 For DR Jack C Rhyan

Final Report on 13-002818 For DR Jack C Rhyan



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

USDA APHIS VS

Fort Collins, CO

Animal Location

Park County MT

Submitter - 2649

DR Jack C. Rhyan

USDA, APHIS, VS

National Wildlife Research Center

4101 La Porte Ave.

Fort Collins, CO 80521

FAX #: 970-266-6138

Phone #: 970-266-6140

Accession Number:

13-002818

Date Collected:

01/09/2013

Date Received:

01/24/2013

Date Completed:

02/27/2013

Collected By:

Rhyan

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: G02 Animal ID: G02 Brucella Case Number: B13-0012 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G03 Animal ID: G03 Brucella Case Number: B13-0013 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G04 **Animal ID:** G04 **Brucella Case Number:** B13-0014 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G06 **Animal ID:** G06 **Brucella Case Number:** B13-0015 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G08 **Animal ID:** G08 **Brucella Case Number:** B13-0016 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G09 **Animal ID:** G09 **Brucella Case Number:** B13-0017 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G10 **Animal ID:** G10 **Brucella Case Number:** B13-0018 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G14 **Animal ID:** G14 **Brucella Case Number:** B13-0019 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G15 **Animal ID:** G15 **Brucella Case Number:** B13-0020 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: G17 **Animal ID:** G17 **Brucella Case Number:** B13-0021 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R01 Animal ID: R01 Brucella Case Number: B13-0022 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R02 Animal ID: R02 Brucella Case Number: B13-0023 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R03 Animal ID: R03 Brucella Case Number: B13-0024 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R04 Animal ID: R04 Brucella Case Number: B13-0025 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R05 Animal ID: R05 Brucella Case Number: B13-0026 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R06 Animal ID: R06 Brucella Case Number: B13-0027 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R07 Animal ID: R07 Brucella Case Number: B13-0028 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R08 Animal ID: R08 Brucella Case Number: B13-0029 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R09 Animal ID: R09 Brucella Case Number: B13-0030 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R11 Animal ID: R11 Brucella Case Number: B13-0031 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R13 Animal ID: R13 Brucella Case Number: B13-0032 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R14 Animal ID: R14 Brucella Case Number: B13-0033 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R15 Animal ID: R15 Brucella Case Number: B13-0034 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R16 Animal ID: R16 Brucella Case Number: B13-0035 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R17 Animal ID: R17 Brucella Case Number: B13-0036 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R18 Animal ID: R18 Brucella Case Number: B13-0037 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R19 Animal ID: R19 Brucella Case Number: B13-0038 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R20 Animal ID: R20 Brucella Case Number: B13-0039 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R21 Animal ID: R21 Brucella Case Number: B13-0040 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R22 Animal ID: R22 Brucella Case Number: B13-0041 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R23 Animal ID: R23 Brucella Case Number: B13-0042 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R24 Animal ID: R24 Brucella Case Number: B13-0043 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R25 Animal ID: R25 Brucella Case Number: B13-0044 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R26 Animal ID: R26 Brucella Case Number: B13-0045 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R27 Animal ID: R27 Brucella Case Number: B13-0046 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R28 Animal ID: R28 Brucella Case Number: B13-0047 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R29 Animal ID: R29 Brucella Case Number: B13-0048 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R30 Animal ID: R30 Brucella Case Number: B13-0049 Specimen Type: BLOOD, SWAB Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R31 **Animal ID:** R31 **Brucella Case Number:** B13-0050 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

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From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: Final Report on 13-002820 For DR Jack C Rhyan
Date: Wednesday, June 19, 2013 10:52:09 AM
Attachments: [13-002820_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:18 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-002820 For DR Jack C Rhyan

Final Report on 13-002820 For DR Jack C Rhyan



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

USDA/APHIS/VS

Fort Collins, CO

Animal Location

Larimer County CO

Submitter - 2649

DR Jack C. Rhyan

USDA, APHIS, VS

National Wildlife Research Center

4101 La Porte Ave.

Fort Collins, CO 80521

FAX #: 970-266-6138

Phone #: 970-266-6140

Accession Number:

13-002820

Date Collected:

Date Received:

01/24/2013

Date Completed:

02/25/2013

Collected By:

McCollum

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 641-10/1 **Animal ID:** 641 **Brucella Case Number:** B13-0051 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Sample: 426-9/19 **Animal ID:** 426 **Brucella Case Number:** B13-0052 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Sample: 393-9/19 **Animal ID:** 393 **Brucella Case Number:** B13-0053 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

Sample: 354-9/19 **Animal ID:** 354 **Brucella Case Number:** B13-0054 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Sample: 017-1/8 **Animal ID:** 017 **Brucella Case Number:** B13-0055 **Specimen Type:** BLOOD, SEMEN **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Semen / Semen

Brucella Isolation Result

No Isolation Made

Semen / Semen

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

To expedite the processing of your sample(s), please include the word "**samples**" in the address on the *ship to* label.
This will assist dock receiving personnel in routing your sample(s).

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Final Report on 13-005136 For DR Patrick Ryan Clarke Ryan
Date: Wednesday, June 19, 2013 10:54:02 AM
Attachments: [13-005136_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:19 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-005136 For DR Patrick Ryan Clarke Ryan

Final Report on 13-005136 For DR Patrick Ryan Clarke Ryan



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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

BQFS - Gonacon Project

Corwin Spring, MT

Animal Location

Park County MT

Submitter - 1961

DR Patrick Ryan Clarke Ryan

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Accession Number:

13-005136

Date Collected:

02/01/2013

Date Received:

02/12/2013

Date Completed:

02/27/2013

Collected By:

Rebecca Frey

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red 03 **Animal ID:** Red 03 **Brucella Case Number:** B13-0061 **Specimen Type:** SWAB, MILK, BLOOD **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Isolate Determined

Milk / Milk

Brucella Isolation Result

Isolate Determined

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Sample: Green 08 **Animal ID:** Green 08 **Brucella Case Number:** B13-0062 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: Green 09 **Animal ID:** Green 09 **Brucella Case Number:** B13-0063 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

To expedite the processing of your sample(s), please include the word "**samples**" in the address on the *ship to* label.
This will assist dock receiving personnel in routing your sample(s).

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Final Report on 13-006974 For DR Patrick Ryan Clarke Ryan
Date: Wednesday, June 19, 2013 10:54:41 AM
Attachments: [13-006974_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:19 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-006974 For DR Patrick Ryan Clarke Ryan

Final Report on 13-006974 For DR Patrick Ryan Clarke Ryan



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Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

Bison Quarantine Feasibility - GonaCon
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Patrick Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number:

13-006974

Date Collected:

02/18/2013

Date Received:

02/26/2013

Date Completed:

03/13/2013

Collected By:

R. Frey

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Grn 15 **Animal ID:** Grn 15 **Brucella Case Number:** B13-0075 **Specimen Type:** SWAB, MILK **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Environmental / Environmental

Brucella Isolation Result

No Isolation Made

Environmental / Environmental

Brucella Isolation Result

No Isolation Made

Environmental / Environmental

Brucella Isolation Result

No Isolation Made

Environmental / Environmental

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

The subsamples listed as 'Environmental' above were labeled as follows: Abortion-Top, Abortion-Bottom, Implant-Top, Implant-Bottom.

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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To expedite the processing of your sample(s), please include the word "**samples**" in the address on the *ship to* label.

This will assist dock receiving personnel in routing your sample(s).

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Final Report on 13-013440 For DR Patrick Ryan Clarke Ryan
Date: Wednesday, June 19, 2013 11:18:33 AM
Attachments: [13-013440_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:20 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-013440 For DR Patrick Ryan Clarke Ryan

Final Report on 13-013440 For DR Patrick Ryan Clarke Ryan



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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

Bison Quarantine Feasibility
, MT

Animal Location

Park County MT

Submitter - 1961

Patrick Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number:

13-013440

Date Collected:

04/11/2013

Date Received:

04/16/2013

Date Completed:

05/06/2013

Collected By:

R. Frey

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red 16 **Animal ID:** Red 16 **Brucella Case Number:** B13-0083 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Milk / Milk Cream

Brucella Isolation Result

Suspect Isolated

Milk / Milk Sediment

Brucella Isolation Result

Suspect Isolated

Swab / Swab- Vaginal

Brucella Isolation Result

Suspect Isolated

Swab / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Feces / Feces

Brucella Isolation Result

Suspect Isolated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

No Isolation Made

Tissue / Placenta

Brucella Isolation Result

Isolate Determined

Brucella Identification Result	Brucella abortus biovar 1
Tissue / Placenta	
Brucella Isolation Result	Suspect Isolated

Only one specimen was chosen for complete identification. Quantification is as follows:

Milk, Cream = >100 cfu

Milk, Sediment = >100 cfu

Swab, Vaginal = TNTC (lawn)

Swab, AT = >100 cfu

Swab, AB = >100 cfu

Swab, AE = TNTC (lawn)

Swab, IT = >100 cfu

Swab IB = >100 cfu

Feces = (>100 cfu heavy contamination)

Placenta = >100 cfu

Placenta = >100 cfu

Sample: Grn 08 Animal ID: Grn 08 Brucella Case Number: B13-0084 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: Grn 09 Animal ID: Grn 09 Brucella Case Number: B13-0085 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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Find our submission forms at: http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml

This is a fillable .pdf. If you would prefer to complete the forms by hand, please write legibly.

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Final Report on 13-013849
Date: Wednesday, June 19, 2013 11:20:09 AM
Attachments: [13-013849_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:20 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-013849

Final Report on 13-013849



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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

Bison Quarantine Herd

Gardiner, MT

Animal Location

Park County MT

Submitter - 2047

MT Department of Livestock

Diagnostic Laboratory Division

19th and Lincoln

PO Box 997

Bozeman, MT 59771-0997

FAX #: 406-994-6344

Phone #: 406-994-4885

Accession Number:

13-013849

Date Collected:

04/11/2013

Date Received:

04/18/2013

Date Completed:

04/25/2013

Collected By:

Dr. Jack Rhyan, (Becky Frey)

Purpose:

General Diagnostic

Referral Number:

8-435-13

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 8-435 **Animal ID:** Red 16 fetus / bison **Brucella Case Number:** B13-0086 **Specimen Type:** Culture **Species:** Bison

Brucella Final Identification

Brucella abortus biovar 1

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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Find our submission forms at: http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml

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From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Final Report on 13-015343 For DR Patrick Ryan Clarke Ryan
Date: Wednesday, June 19, 2013 11:22:09 AM
Attachments: [13-015343_1.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, June 19, 2013 10:21 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Subject: Final Report on 13-015343 For DR Patrick Ryan Clarke Ryan

Final Report on 13-015343 For DR Patrick Ryan Clarke Ryan



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FINAL REPORT

Laboratory Test Report

***** This is a confidential report intended for official use only. *****

Owner

Bison Quarantine

Corwin Springs, MT

Animal Location

Park County MT, US

Submitter - 1961

Patrick Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Accession Number:

13-015343

Date Collected:

04/22/2013

Date Received:

04/30/2013

Date Completed:

05/14/2013

Collected By:

RK Frey / R. Clarke

Purpose:

Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 3R21 **Animal ID:** 3R21 **Brucella Case Number:** B13-0089 **Specimen Type:** BLOOD, SWAB **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: Red 21 **Animal ID:** Red 21 **Brucella Case Number:** B13-0090 **Specimen Type:** SWAB, BLOOD, MILK **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Exudate / Exudate- Vaginal

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Swab / Swab- Vaginal

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Swab / Swab, Placenta

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Feces / Feces

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

All suspect and confirmed positive specimens were too numerous to count.

Sample: Green 09 **Animal ID:** Green 09 **Brucella Case Number:** B13-0091 **Specimen Type:** SWAB, BLOOD, MILK **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Tissue / Placenta

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Feces / Feces

Brucella Isolation Result

Contaminated

Milk / Milk Cream

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Milk / Milk Sediment

Brucella Isolation Result

Suspect Isolated

Fetus / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Brucella Identification Result

Not a Brucella species

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab, Environmental / Swab- Not Identified

Brucella Isolation Result

Suspect Isolated

Swab / Swab- Vaginal

Brucella Isolation Result

Suspect Isolated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Blood / Blood- Enriched in TSB

Brucella Isolation Result

Contaminated

All suspect and confirmed positive specimens were too numerous to count.**Results authorized by:** Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**Help Us Help You**

(This new section will be updated periodically with tips for submitters.)

Find our submission forms at: http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml

This is a fillable .pdf. If you would prefer to complete the forms by hand, please write legibly.

From: [Rhyan, Jack C - APHIS](#)
To: [Eisemann, John D - APHIS](#); [Stephens, Stephanie H - APHIS](#)
Cc: [Herriott, Donald E - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Bundy, Mildred O - APHIS](#); [Nelson, Janell - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: FW: FOIA #2012-APHIS-01625-F
Date: Wednesday, February 29, 2012 11:42:25 AM
Attachments: [APHIS FOIAappeal GonaCon 2-23-2012.pdf](#)
[12-01625 \(Response\) Letter.pdf](#)
[APHIS FOIAreq GonaCon 2-2012.pdf](#)
Importance: High

John and Stephanie,

I don't know if you've seen this or not. I don't think we have any of the documents regarding the EPA GonaCon permit. Can you please send Mildred what you have?

Thanks,

Jack

From: Nelson, Janell - APHIS
Sent: Friday, February 24, 2012 12:32 PM
To: McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Cc: Bundy, Mildred O - APHIS; Camp, Celeste - APHIS
Subject: FOIA #2012-APHIS-01625-F
Importance: High

Drs. Herriott, McCluskey, Clarke, Rhyan, & Nol:

The attached FOIA request was received by e-mail last week. We and the FOIA Office informed the requestor that the information requested was available online during the comment period and provided the website for their convenience. They have appealed that response, now requesting all documents ... etc as outlined in the *APHIS FOIAappeal GonaCon 2-23-2012.pdf* attachment. I know that we provided some documents regarding the GonaCon study in the response to FOIA 11-548, but I am not familiar enough with the parts of the study to know if these materials are the complete response to this new request.

As you are aware, we now have five (5) work days to:

- search for the appropriate records (paper and electronic),
- create copies of the records, and
- deliver them and the completed Request for Document Search form to the FOIA liaison (Mildred Bundy -- her address is listed below).

Do not create new documents (e.g. lists, tables, any kind of compilation from records) in response to FOIA requests. The FOIA office will redact any Privacy Act-protected information from the records you provide to Ms. Bundy. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson

Staff Assistant, VS Western Region

970-494-7400

-----Original Message-----

From: Camp, Celeste - APHIS

Sent: Thursday, February 23, 2012 11:30 AM

To: Nelson, Janell - APHIS
Subject: FW: FOIA Appeal #2012-APHIS-01625-F
Importance: High

Janell, would you please give me a call regarding this? It appears, we did not provide him with everything he's asking for.

Celeste Camp
Assistant Director
FOIA/PA Office
Legislative and Public Affairs
(301) 851-4057

-----Original Message-----

From: Taylor, Lyndia F - APHIS
Sent: Thursday, February 23, 2012 1:19 PM
To: Camp, Celeste - APHIS
Subject: FW: FOIA Appeal #2012-APHIS-01625-F
Importance: High

-----Original Message-----

From: Buffalo Field Campaign [<mailto:BFC-Media@wildrockies.org>]
Sent: Thursday, February 23, 2012 12:20 PM
To: Taylor, Lyndia F - APHIS; Boyd, Shirley A - APHIS
Cc: Herriott, Donald E - APHIS; (b) (6) [gmail.com](#); r8foia@epa.gov
Subject: FOIA Appeal #2012-APHIS-01625-F
Importance: High

Dear USDA-APHIS FOIA Office,

Attached is a FOIA Appeal in response to APHIS's response
(2012-APHIS-01625-F) to my original FOIA request dated February 22, 2102.

I have also attached my original FOIA request, the APHIS response, as well as a completely irrelevant document that APHIS included in response to my original request.

A hard copy of this appeal, along with a copy of our original FOIA request, will be sent via traditional mail to your office.

I appreciate your expedited response to this important request.

Sincerely,
Stephany J. Seay

--

Media & Outreach
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
bfc-media@wildrockies.org
<http://www.buffalofieldcampaign.org>

*** BFC is the only group working in the field every day in defense of the last wild buffalo population in the U.S. ***



BUFFALO FIELD CAMPAIGN
P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
406-646-0070

bfc-media@wildrockies.org * <http://www.buffalofieldcampaign.org>

February 23, 2012

Administrator
Animal & Plant Health Inspection Service
Ag Box 3401
Washington, DC 20250-3401

RE: APPEAL OF FEDERAL FREEDOM OF INFORMATION ACT REQUEST RESPONSE #2012-APHIS-01625-F

Dear APHIS FOIA Administrator,

This is an appeal under the Freedom of Information Act.

On February 22, 2012 I requested documents under the Freedom of Information Act. My request was assigned the following identification number: 2012-APHIS-01625-F. On February 22, 2012, I received a response to my request in a letter signed by Tonya G. Woods, Director of USDA-APHIS Freedom of Information & Privacy Act. I appeal the denial of my request. A copy of my FOIA request and the agency determination, which is the subject of this appeal, is attached for your convenience. I have also attached a completely irrelevant document that your office included in their response to my original FOIA request.

Buffalo Field Campaign believes that Ms. Woods misinterpreted the request and that this information is urgently needed. We asked for the supporting documentation: **the records, documentation, permits, emails, and other information surrounding the USDA-APHIS request to EPA to use GonaCon for experimental use on bison.** Instead of sending us the requested information, your office they referenced us to the EA link, which we already have and which is lacking the information we are requesting. The EA link that you sent simply downloads the EA. The EA does not contain the supporting records, documentation, permits, e-mails, or other information surrounding the request by APHIS to EPA to use GonaCon on bison.

Buffalo Field Campaign asks that this request be expedited as these documents are critical to our ability to meaningfully comment on the APHIS EA, "Evaluation of GonaCon in Bison", for which the public comment deadline is February 25, 2011. Buffalo Field Campaign requests that *all records and documentation be provided in electronic form via email to bfc-media@wildrockies.org as well as on a CD, so as to reduce time, cost and waste.* Disclosure of the documents I requested is in the public interest because it is likely to contribute significantly to public understanding of the operations or activities of the government and is not primarily in my commercial interest.

Sincerely,


Stephany J. Seay
Buffalo Field Campaign

Cc:

- Daniel C. Snyder, Law Offices of Charles M. Tebbutt, P.C
- U.S. Environmental Protection Agency FOIA Office
- USDA-APHIS Veterinary Services, Dr. Don Herriott, EA Agency Contact

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United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Legislative and
Public Affairs

Freedom of
Information

4700 River Road
Unit 50
Riverdale, MD
20737-1232

February 22, 2012

Stephany Seay
Post Office Box 957
West Yellowstone, Montana, 59758

Dear Stephany Seay:

This is in response to your February 21, 2012, Freedom of Information Act (FOIA) request for the records surrounding APHIS's official comments to the Environmental Protection Agency (EPA) concerning the use of GonaCon for experimental use on Bison. Your request was received in this office on February 21, 2012, and assigned tracking number 2012-APHIS-01625-F.

The Program office advises that the information you are seeking is publicly available at the following website:

http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/gnrh_ea.pdf

You may appeal our adequacy of search. If you choose to appeal, your appeal must be in writing and received within 45 days of the date of this letter. Please send your appeal to:

Administrator
Animal and Plant Health Inspection Service
Ag Box 3401
Washington, DC 20250-3401

If you should appeal, please refer to tracking number 2012-APHIS-01625-F in your appeal letter and add the words "FOIA Appeal" to the front of the envelope. To assist the Administrator in reviewing your appeal, provide specific reasons for the appeal.

Because the cost to process your request is less than \$25.00, all fees have been waived. If you have any questions, please contact Ms. Lyndia Taylor of my staff at (301) 851-4042.

Sincerely,


Tonya G. Woods
Director

Freedom of Information & Privacy Act
Legislative and Public Affairs



BUFFALO FIELD CAMPAIGN
P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
406-646-0070

bfc-media@wildrockies.org * <http://www.buffalofieldcampaign.org>

February 22, 2012

Tonya Woods, FOIA/PA Officer
USDA-Animal & Plant Health Inspection Service
4700 River Road, Unit 50
Riverdale, MD 20737-1232

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Dear Ms. Woods,

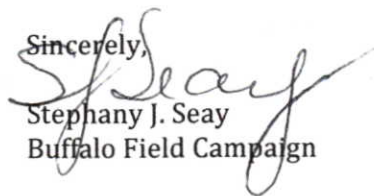
On behalf of Buffalo Field Campaign, a Montana-based wild bison advocacy group representing tens of thousands of concerned citizens in Montana, throughout the United States and around the globe, working in defense of America's last wild bison population, the Yellowstone herds, I file a Freedom of Information Act request.

This request pertains to the Environmental Assessment released by USDA-Animal & Plant Health Inspection Service, regarding the "Experimental use of GonaCon in Bison." APHIS has allowed for a very brief public comment period on an issue of great concern, for which APHIS failed to 1) adequately notify the public of the availability of the EA; 2) allow an adequate comment period; 3) disclose critical information related to the proposed study. The APHIS EA lacks critical documentation necessary for an understanding of the proposed study, and for meaningful, educated comments from the public. I contacted the Environmental Protection Agency Insecticide-Rodenticide Branch, as well as USDA-APHIS EA contact Dr. Don Herriott, requesting the information we seek, yet was told that a FOIA request needed to be submitted. On behalf of Buffalo Field Campaign, I hereby submit that request with urgency.

Buffalo Field Campaign requests Under the Freedom of Information Act Request, 5 U.S.C. § 552, the records, documentation, permits, emails, and other information surrounding the USDA-APHIS request to EPA to use GonaCon for experimental use on bison.

Buffalo Field Campaign asks that this request be expedited as these documents are critical to our ability to meaningfully comment on the APHIS EA, "Experimental use of GonaCon in Bison", for which the public comment deadline is Friday, February 25, 2011. Buffalo Field Campaign requests that *all records and documentation be provided in electronic form via email to bfc-media@wildrockies.org as well as on a CD, so as to reduce time, cost and waste.*

Sincerely,


Stephany J. Seay
Buffalo Field Campaign

Cc:

- Daniel C. Snyder, Law Offices of Charles M. Tebbutt, P.C
- U.S. Environmental Protection Agency FOIA Office
- USDA-APHIS Veterinary Services, Dr. Don Herriott, EA Agency Contact



UNITED STATES DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C. 20250

OFFICE OF THE SECRETARY OF AGRICULTURE

WASHINGTON, D. C. 20250

FOR INFORMATION OF THE SECRETARY OF AGRICULTURE

DATE: 10/10/68

TO: Mr. [Name]

FROM: Mr. [Name]

SUBJECT: [Subject]

RE: [Subject]

1. [Text]

2. [Text]

3. [Text]

4. [Text]

5. [Text]

6. [Text]

7. [Text]

8. [Text]

9. [Text]

10. [Text]

11. [Text]

12. [Text]

From: [Eisemann, John D - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: FOIA Search - 12-00575
Date: Wednesday, April 11, 2012 11:22:04 AM
Attachments: [1 - 2012-01161 Request 1-6-12.pdf](#)

FYI

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

This electronic message contains information generated by the USDA solely for the intended recipients. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the violator to civil or criminal penalties. If you believe you have received this message in error, please notify the sender and delete the email immediately.

From: Romines, Janean - APHIS
Sent: Wednesday, April 11, 2012 8:06 AM
To: Eisemann, John D - APHIS
Cc: Clark, Larry - APHIS; Tobin, Mark E - APHIS; Deliberto, Thomas J - APHIS; Freeman, Nancy - APHIS
Subject: FW: FOIA Search - 12-00575

Hi John-

Please see the email below regarding responsive records to the attached request. WS did not originally receive this request; it went to VS and they provided a negative response. VS is saying this was a WS study. Can you please check to see if you have records that would be responsive?

Also, I will be on a conference call tomorrow with APHIS FOIA and OGC regarding the release of the GonaCon formulation. I have requested that the information be withheld under FIFRA. EPA does use the FIFRA Statute to withhold confidential information and in a 2010 FOIA request we used this same statute to withhold the GonaCon information; however, there seems to be a question on whether or not we can protect this because we are not a private entity. The government does not get many privacy rights. I will keep you posted.

Thanks for doing a search for the records and returning the records (if found) or a negative reply. Please call if you have questions.

Janean Romines

Staff Officer/Wildlife Biologist

Operational Support Staff
USDA/APHIS/Wildlife Services
4700 River Road
Unit 87, Rm. 2B-08.2
Riverdale, MD 20737
Off: 301.851.3996
Cell: (b) (6)
Fax: 301.734.5157

From: Camp, Celeste - APHIS
Sent: Wednesday, April 11, 2012 7:04 AM
To: Doerr, Michael R - APHIS; Hamm, Shannon R - APHIS; Romines, Janean - APHIS
Cc: Bundy, Mildred O - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS
Subject: RE: FOIA Search - 12-00575

Janean, would you please take a look at the attached request and let me know if WS would have the requested information? As you can see from VS' response, they do not have any responsive records, but has indicated this was WS' study.

Celeste

From: Doerr, Michael R - APHIS
Sent: Tuesday, April 10, 2012 5:55 PM
To: Hamm, Shannon R - APHIS
Cc: Bundy, Mildred O - APHIS; Camp, Celeste - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS
Subject: Re: FOIA Search - 12-00575

It is a Wildlife Services study.

Michael Doerr

Chief Operating Officer

USDA-APHIS-Veterinary Services

On Apr 10, 2012, at 5:49 PM, "Hamm, Shannon R - APHIS"
<Shannon.R.Hamm@aphis.usda.gov> wrote:

<image001.jpg>

Did you not conduct a cooperative agreement or contract for the study?

From: Bundy, Mildred O - APHIS

Sent: Tuesday, April 10, 2012 2:17 PM

To: Hamm, Shannon R - APHIS; Camp, Celeste - APHIS

Cc: Doerr, Michael R - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS;
Bundy, Mildred O - APHIS

Subject: FOIA Search - 12-00575

Pursuant to appropriation questions for the Bison Quarantine Feasibility Study, there wasn't a earmark for the Bison Quarantine Feasibility Study, that project is funded as part of the brucellosis program. There is no separate budget for that project, therefore there is no way to identify a "balance of funds" for the project and we did not separately track spending on that project.

12-01161



Darrell Geist
<z@wildrockies.org>
01/06/2012 02:19 PM

To FOIA Officer/MD/APHIS/USDA
cc <z@wildrockies.org>, Patrick R Clarke/MT/APHIS/USDA
bcc
Subject JANUARY 6 2012 FREEDOM OF INFORMATION ACT
REQUEST

1 attachment



P1D1278DE 6 1 2.png

FOIA Request # 12-01161
Date Rec'd 1/6/2012
Date Due 1/6/2012
Assigned to Robbie
Category All other
Search VS



BUFFALO FIELD CAMPAIGN

P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
(406) 646-0070 PHONE (406) 646-0071 FAX
<http://www.buffalofieldcampaign.org>
buffalo@wildrockies.org

January 6, 2012

Tonya Woods, FOIA/PA Officer
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 50
Riverdale, MD 20737-1232
Tel. 301-734-5267
Fax 301-734-5941
Email: FOIA.Officer@aphis.usda.gov

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Ms. Woods:

Pursuant to the federal Freedom of Information Act (5 U.S.C. 552 et. seq.), Buffalo Field Campaign is filing this request for information.

Buffalo Field Campaign is a 501(c) (3) non-profit, public interest, grassroots media-based organization, which provides news reports directly to thousands of supporters which include concerned American citizens, and people from around the globe, as well as to regional, national and international media.

We would prefer an electronic copy of this information on CD but we would be happy to get a paper copy of anything that is not available electronically.

We request the following documentation from USDA APHIS:

1. Brucella Genotyping Reports (final, preliminary, draft) generated by APHIS during calendar years 2010 and 2011 for incidents or suspected incidents of *brucella abortus* infection in elk, bison and cattle in Montana, Idaho, and Wyoming.

As you know, the Freedom of Information Act (FOIA) provides that if portions of a document are exempt from release, the remainder must be segregated and disclosed. We expect to receive all non-exempt portions of the documents that we have requested, and ask that you justify any deletions by reference to specific exemptions allowed under the FOI Act. The Buffalo Field Campaign reserves the right to appeal a decision to withhold any materials.

We hereby request a fee waiver for all search and duplication fees under the FOIA regulations [5 U.S.C. Sec. 552 (a) (4) (A) and 36 CFR 2.19(c) (1)]. The information requested will benefit the citizens of the United States and is for the purpose of public education and to encourage public debate on important policy issues. The requested information will be made available to the public through Buffalo Field Campaign's central office and/or our website.

Information available through the office and website is used in press conferences and releases, television and radio interviews, and regional and national publications, and reaches a significant number of individuals nationwide, including through the following news sources: New York Times, Los Angeles Times, Washington Post, CNN, CBS, ABC, NBC, Headline News, London Times, UK Guardian, Japanese and German TV, National Geographic, PBS, Associated Press (nationally syndicated), Reuters (internationally syndicated), Planet Green Discovery Channel, Examiner, Indian Country Today, News from Indian Country, Bozeman Daily Chronicle, Helena Independent Record, Billings Gazette, Missoulian, Great Falls Tribune, West Yellowstone News, Livingston Enterprise, Montana Pioneer, Montana Standard, Flathead Beacon, Missoula Independent, Big Sky Weekly, Montana Public Radio, Pacifica Radio Stations, WBAI First Voices Indigenous Radio, KBZK-TV Bozeman, KXLF-TV Butte, ABC Montana, NBC Montana, CBS Montana, KGNU Colorado, Fox News Channel 8 Cleveland, Montana News Casper Star Tribune, Planet Jackson Hole, Jackson Hole News & Guide, Jackson Hole Weekly, Island Park News, Salt Lake Tribune, Powell Tribune, Ag Information Network, Idaho Statesman, Huffington Post, Word Press, New West, Yahoo! News, AlterNet, Mother Jones, Prairie Star, The Republic, Environmental News Service, Earth First! Journal, Mother Nature Network, CounterPunch, Animal People, Independent Media, multiple blogs and online news resources.

The language of the FOIA clearly indicates that Congress intended fees not to be a barrier to private individuals or public

interest organizations seeking access to government records. In addition, the legislative history of the FOIA fee waiver language indicates that Congress intended a liberal interpretation of the phrase "primarily benefiting the public." This suggests that all fees are to be waived whenever the release of information contributes to public debate on important policy issues. This has been affirmed by the US Court of Appeals for the District of Columbia, in *Better Government Association v. Department of State*, 780 F. 2d 86 (D.C. Cir. 1986). In that case, the court found that under the FOIA, Congress had explicitly recognized the need for non-profit organizations to have free access to government documents and those government agencies cannot impair this free access by charging duplication or search for FOIA information requests. *Id.* at 89.

I appreciate your help and prompt response. Thank you for your time.

Sincerely,

/s/
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
<http://www.buffalofieldcampaign.org>

From: [Eisemann, John D - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: FOIA Search - 12-00575
Date: Wednesday, April 11, 2012 11:22:24 AM

FYI

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

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From: Eisemann, John D - APHIS
Sent: Wednesday, April 11, 2012 8:51 AM
To: Romines, Janean - APHIS
Cc: Clark, Larry - APHIS; Tobin, Mark E - APHIS; Deliberto, Thomas J - APHIS; Freeman, Nancy - APHIS
Subject: RE: FOIA Search - 12-00575

Janean, I looked at the FOIA request. Buffalo Fields Campaign has made a very specific request:

1. *Brucella* Genotyping Reports (final, preliminary, draft) generated by APHIS during calendar years 2010 and 2011 **for incidents or suspected incidents of brucella abortus infection in elk, bison and cattle in Montana, Idaho, and Wyoming.**

To my knowledge, NWRC does not have any of these records. If this information was collected by APHIS, I believe it would have been done by Veterinary Services.

I spoke with Nancy Freeman this morning and she indicated that she will follow up with you this morning.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

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Hi John-

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Also, I will be on a conference call tomorrow with APHIS FOIA and OGC regarding the release of the GonaCon formulation. I have requested that the information be withheld under FIFRA. EPA does use the FIFRA Statute to withhold confidential information and in a 2010 FOIA request we used this same statute to withhold the GonaCon information; however, there seems to be a question on whether or not we can protect this because we are not a private entity. The government does not get many privacy rights. I will keep you posted.

Thanks for doing a search for the records and returning the records (if found) or a negative reply. Please call if you have questions.

Janean Romines

Staff Officer/Wildlife Biologist
Operational Support Staff
USDA/APHIS/Wildlife Services
4700 River Road
Unit 87, Rm. 2B-08.2
Riverdale, MD 20737
Off: 301.851.3996
Cell: (b) (6)
Fax: 301.734.5157

From: Camp, Celeste - APHIS
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To: Doerr, Michael R - APHIS; Hamm, Shannon R - APHIS; Romines, Janean - APHIS
Cc: Bundy, Mildred O - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS

Subject: RE: FOIA Search - 12-00575

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Celeste

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To: Hamm, Shannon R - APHIS
Cc: Bundy, Mildred O - APHIS; Camp, Celeste - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS
Subject: Re: FOIA Search - 12-00575

It is a Wildlife Services study.

Michael Doerr

Chief Operating Officer

USDA-APHIS-Veterinary Services

On Apr 10, 2012, at 5:49 PM, "Hamm, Shannon R - APHIS"
<Shannon.R.Hamm@aphis.usda.gov> wrote:

<image001.jpg>

Did you not conduct a cooperative agreement or contract for the study?

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Sent: Tuesday, April 10, 2012 2:17 PM
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Cc: Doerr, Michael R - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search - 12-00575

Pursuant to appropriation questions for the Bison Quarantine Feasibility Study, there wasn't a earmark for the Bison Quarantine Feasibility Study, that project is funded as part of the brucellosis program. There is no separate budget for that project, therefore there is no way to identify a "balance of funds" for the project and we did not separately track spending on that project.

From: [Frey, Rebecca K \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#)
Subject: Fw: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011
Date: Friday, June 10, 2011 8:15:22 AM
Attachments: [564.pdf](#)

Hi,
I think our project proposal will satisfy this, though I was wondering if you had anything on budget for this. I have never seen anything, other than knowing what the leases are.
Thanks,
Becky
Becky Frey

From: Janell R Nelson
Sent: 06/09/2011 10:58 PM GMT
To: Patrick Clarke; Jack Rhyan; Rebecca Frey
Cc: Janell Nelson
Subject: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Drs. Clarke, Rhyan & Frey:

We have received the FOIA search request attached below.
As you are aware, we now have five (5) work days to:
-search for the appropriate records (paper and electronic),
-create copies of the records, and
-deliver them and the completed Request for Document Search form to the WRO (to my attention).

The FOIA office will redact any Privacy Act-protected information from the records we provide to the FOIA Liaison. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise me by e-mail when the response documents are en route to this office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O (APHIS)
Sent: Thursday, June 09, 2011 12:39 PM
To: Nelson, Janell (APHIS)
Subject: New Request: FOIA 11-564

Hi Janelle: Hope all is well.

11- 564

Tamara
VS
Individual



Max Coats
(b) (6) @hughes.net>
06/07/2011 01:41 AM

To foia.officer@aphis.usda.gov
cc
bcc

Subject Request for Information - Bison Project

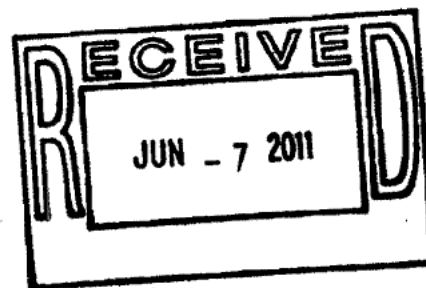
Please consider the following as an official FOIA Request:

I read in the USAHA Alerts about a proposed project involving Yellowstone bison and temporary sterilization. I seek specific information detailing the protocol, total project cost estimates and the logic presented to support the project.

In addition I would request information advising who the principal investigators are to be. I was advised by the Director, Western Region, Veterinary Services to forward my request to you.

Thank you for your response

Max E. Coats Jr. DVM, MS
Cell Phone (b) (6)
E.-Mail (b) (6) @hughes.net



JUL - 6 2011

From: [Linfield, Thomas F - APHIS](#)
To: [Quance, Christine R - APHIS](#); [Higgins, James A - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Herriott, Donald E - APHIS](#); [McCluskey, Brian J - APHIS](#); [Frey, Rebecca K - APHIS](#); [Nelson, Janell - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Tuesday, February 21, 2012 11:18:48 AM
Attachments: [image001.png](#)
[12-01161.pdf](#)

Chris, James:

Attached is the actual FOIA Request – and the following are the Genotyping reports requested:

*“1.1 Brucella Genotyping Reports (final, preliminary, draft)
generated by APHIS during calendar years 2010 and 2011 for incidents or
suspected incidents of brucella abortus infection in elk, bison and cattle in Montana, Idaho,
and
Wyoming.”*

I have received the final MT Genotyping reports during the requested time-frame from James Higgins, however, I have not received all reports (ID or WY) - not sure what other reports may have been generated in CY 2010 and CY 2011 for “suspected incidents of brucella abortus infection in elk, bison and cattle” in Idaho and Wyoming... as such, may be more appropriate for James to forward all reports. I believe all personal identifying information (names, ranch names, etc) should be redacted from all reports – not sure if Mildred performs redactions prior to forwarding the reports to the requester.

Also, there were some needed corrections to the Snowcrest report, and believe those corrections should be incorporated into an amended final report before forwarding.

I would be happy to work with James re: the MT reports, including the suggested edits to the Snowcrest report. I discussed this issue with Dr. Herriott – told him I could forward Mildred the MT reports, however, may be more appropriate coming from James, since he was the author of all reports

Thomas F.T. Linfield, DVM

AVIC-Montana
USDA-APHIS-Veterinary Services
208 N. Montana Ave; Suite 101
Helena, MT 59601
(406) 449-2220
(406) 449-5439 FAX
Thomas.F.Linfield@aphis.usda.gov

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 21, 2012 10:53 AM
To: Quance, Christine R - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS; Bundy, Mildred O - APHIS; Nelson, Janell - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J -

APHIS; Herriott, Donald E - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Chris,

We've all been talking about you but I thought maybe we should talk with you. Hmm. As you can see, there was a FOIA request for which we think you have the needed info.

We assumed the FOIA folks were talking with you but apparently not. So now, just before they haul us off to federal prison for being so tardy, I thought maybe you should be given the opportunity to save us all.

Have you heard of this? Can you provide this info?

Is there somewhere else to get it?

I'll send you the original request if I still have it.

Jack

From: Bundy, Mildred O - APHIS

Sent: Tuesday, February 21, 2012 5:29 AM

To: Nelson, Janell - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

FYI: I never received anything from NVSL.

From: Nelson, Janell - APHIS

Sent: Thursday, January 26, 2012 3:39 PM

To: Bundy, Mildred O - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Mildred:

By my calendar, it is one week overdue; but we believed that your e-mail from 1/12/2012 stated the information from NVSL was sufficient to answer the request. Please clarify. We are not trying to withhold anything, we just thought it had been taken care of by the folks with the original documents.

Janell

Yes, it will fulfill the request. Thanks

From: Clarke, Patrick R. - APHIS

Sent: Wednesday, January 11, 2012 10:27 AM

To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: Bundy, Mildred O - APHIS

Sent: Thursday, January 26, 2012 1:28 PM

To: Clarke, Patrick R. - APHIS; Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

THIS CASE IS ALMOST 2 WEEKS OVERDUE!!!!!!!!!! Can someone please forward as soon as possible.
Thanks

From: Clarke, Patrick R. - APHIS

Sent: Wednesday, January 11, 2012 10:27 AM

To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyan:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

- search** for the appropriate records (paper and electronic),
- create copies** of the records, and
- deliver them** and the completed Request for Document Search form to the FOIA liaison (**Mildred Bundy** -- her address is listed below).

Do not create new documents (e.g. lists, tables, any kind of compilation from records) in response to FOIA requests. The FOIA office will redact any Privacy Act-protected information from the records you provide to Ms. Bundy. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR
REQUEST #: 2012-APHIS-01161F

REQUESTER: GEIST
DUE TO FOIA: 1/18/12

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: mildred.bundy@aphis.usda.gov, if sending by mail, send to USDA, APHIS, MILDRED BUNDY, 4700 Riverdale Road, Riverdale, MD 20737.

SEARCH START DATE:

Search time* (clerical): _____

Search time* (professional): _____

***Does not include photocopying time:** _____

Review time (professional): _____

Search conducted by:

Name

Title

Office and Phone

Missing Document Explanation/Special Notes:

12-01161



Darrell Geist
<z@wildrockies.org>
01/06/2012 02:19 PM

To FOIA Officer/MD/APHIS/USDA
cc <z@wildrockies.org>, Patrick R Clarke/MT/APHIS/USDA
bcc
Subject JANUARY 6 2012 FREEDOM OF INFORMATION ACT
REQUEST

1 attachment



P1D1278DE 6 1 2.png

FOIA Request # 12-01161
Date Rec'd 1/6/2012
Date Due 1/6/2012
Assigned to Robbie
Category All other
Search VS



BUFFALO FIELD CAMPAIGN

P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
(406) 646-0070 PHONE (406) 646-0071 FAX
<http://www.buffalofieldcampaign.org>
buffalo@wildrockies.org

January 6, 2012

Tonya Woods, FOIA/PA Officer
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 50
Riverdale, MD 20737-1232
Tel. 301-734-5267
Fax 301-734-5941
Email: FOIA.Officer@aphis.usda.gov

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Ms. Woods:

Pursuant to the federal Freedom of Information Act (5 U.S.C. 552 et. seq.), Buffalo Field Campaign is filing this request for information.

Buffalo Field Campaign is a 501(c) (3) non-profit, public interest, grassroots media-based organization, which provides news reports directly to thousands of supporters which include concerned American citizens, and people from around the globe, as well as to regional, national and international media.

We would prefer an electronic copy of this information on CD but we would be happy to get a paper copy of anything that is not available electronically.

We request the following documentation from USDA APHIS:

1. Brucella Genotyping Reports (final, preliminary, draft) generated by APHIS during calendar years 2010 and 2011 for incidents or suspected incidents of *brucella abortus* infection in elk, bison and cattle in Montana, Idaho, and Wyoming.

As you know, the Freedom of Information Act (FOIA) provides that if portions of a document are exempt from release, the remainder must be segregated and disclosed. We expect to receive all non-exempt portions of the documents that we have requested, and ask that you justify any deletions by reference to specific exemptions allowed under the FOI Act. The Buffalo Field Campaign reserves the right to appeal a decision to withhold any materials.

We hereby request a fee waiver for all search and duplication fees under the FOIA regulations [5 U.S.C. Sec. 552 (a) (4) (A) and 36 CFR 2.19(c) (1)]. The information requested will benefit the citizens of the United States and is for the purpose of public education and to encourage public debate on important policy issues. The requested information will be made available to the public through Buffalo Field Campaign's central office and/or our website.

Information available through the office and website is used in press conferences and releases, television and radio interviews, and regional and national publications, and reaches a significant number of individuals nationwide, including through the following news sources: New York Times, Los Angeles Times, Washington Post, CNN, CBS, ABC, NBC, Headline News, London Times, UK Guardian, Japanese and German TV, National Geographic, PBS, Associated Press (nationally syndicated), Reuters (internationally syndicated), Planet Green Discovery Channel, Examiner, Indian Country Today, News from Indian Country, Bozeman Daily Chronicle, Helena Independent Record, Billings Gazette, Missoulian, Great Falls Tribune, West Yellowstone News, Livingston Enterprise, Montana Pioneer, Montana Standard, Flathead Beacon, Missoula Independent, Big Sky Weekly, Montana Public Radio, Pacifica Radio Stations, WBAI First Voices Indigenous Radio, KBZK-TV Bozeman, KXLF-TV Butte, ABC Montana, NBC Montana, CBS Montana, KGNU Colorado, Fox News Channel 8 Cleveland, Montana News Casper Star Tribune, Planet Jackson Hole, Jackson Hole News & Guide, Jackson Hole Weekly, Island Park News, Salt Lake Tribune, Powell Tribune, Ag Information Network, Idaho Statesman, Huffington Post, Word Press, New West, Yahoo! News, AlterNet, Mother Jones, Prairie Star, The Republic, Environmental News Service, Earth First! Journal, Mother Nature Network, CounterPunch, Animal People, Independent Media, multiple blogs and online news resources.

The language of the FOIA clearly indicates that Congress intended fees not to be a barrier to private individuals or public

interest organizations seeking access to government records. In addition, the legislative history of the FOIA fee waiver language indicates that Congress intended a liberal interpretation of the phrase "primarily benefiting the public." This suggests that all fees are to be waived whenever the release of information contributes to public debate on important policy issues. This has been affirmed by the US Court of Appeals for the District of Columbia, in *Better Government Association v. Department of State*, 780 F. 2d 86 (D.C. Cir. 1986). In that case, the court found that under the FOIA, Congress had explicitly recognized the need for non-profit organizations to have free access to government documents and those government agencies cannot impair this free access by charging duplication or search for FOIA information requests. *Id.* at 89.

I appreciate your help and prompt response. Thank you for your time.

Sincerely,

/s/
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
<http://www.buffalofieldcampaign.org>

From: [Clarke, Patrick R. - APHIS](#)
To: [Herriott, Donald E - APHIS](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: FREEDOM OF INFORMATION ACT REQUEST
Date: Thursday, October 20, 2011 11:15:18 AM
Attachments: [P1D1278DE 6.png](#)

Looks like a FOIA request is in the pipeline for the BQFS.

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

From: Darrell Geist [mailto:z@wildrockies.org]
Sent: Thursday, October 20, 2011 11:00 AM
To: FOIA Officer
Cc: z@wildrockies.org; Clarke, Patrick R. - APHIS
Subject: FREEDOM OF INFORMATION ACT REQUEST



BUFFALO FIELD CAMPAIGN
P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
(406) 646-0070 PHONE (406) 646-0071 FAX
<http://www.buffalofieldcampaign.org>
buffalo@wildrockies.org

October 20, 2011

Tonya Woods, FOIA/PA Officer
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 50
Riverdale, MD 20737-1232
Tel. 301-734-5267
Fax 301-734-5941
Email: FOIA.Officer@aphis.usda.gov

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Ms. Woods:
Pursuant to the federal Freedom of Information Act (5 U.S.C. 552 et. seq.), Buffalo Field Campaign is filing this request for information.

Buffalo Field Campaign is a 501(c) (3) non-profit, public interest, grassroots media-based organization, which provides news reports directly to thousands of supporters which include concerned American citizens, and tourists from around the globe, as well as to regional, national and international media.

We would prefer an electronic copy of this information but we would be happy to get a paper copy of anything that is not available electronically. We request the following documentation from USDA APHIS:

1. Records that disclose the status of bison captured inside Yellowstone National Park for consignment to USDA APHIS' quarantine feasibility study, specifically those records that account for births and deaths and causes for each death recorded of bison in quarantine.

According to publicly available records provided by USDA APHIS and Montana Fish, Wildlife & Parks:

1st cohort captured inside Yellowstone National Park and trucked to Corwin Springs quarantine in April 2005 with additional buffalo captured for quarantine in 2006.

- + 17 calves trucked in.
- + 85 calves trucked in.
- 3 buffalo sero-converted and were euthanized.
- 4 buffalo sero-converted and were euthanized.
- 2 of 3 "suspect" buffalo were euthanized.
- + 21 calves born in captivity 2008.
- 5 calves died at birth: 4 stillborn from dystocia, 1 rejected by mother.
- 2 mothers died after giving birth.
- 2 calves euthanized after mothers died.
- + 30 calves born in captivity 2009.
- 48 randomly selected buffalo were slaughtered "to determine latent infection. All were culture negative."

1st cohort of 87 buffalo - 33 adult females, 8 adult bulls, 16 yearlings, and 30 calves - trucked to Turner Enterprise Inc.'s Green Ranch in Montana February 17-18, 2010.

- + 21 calves born in captivity 2010.
- 4 buffalo died total: 1 orphaned calf died shortly after transport; yearling died from meningoencephalitis; 5 year old broke a leg; newborn calf died umbilical cord infection; 2 year old female struck by lightning.
- + 40 calves born in captivity 2011.

Buffalo in quarantine on Turner's Green Ranch: 143.

2nd cohort of 112 buffalo captured inside Yellowstone National Park for consignment to quarantine winter 2008.

- + 112 calves trucked in.
- 27 buffalo sero-converted and were euthanized.
- 4 buffalo died; 2 from "unknown causes" and 2 from "trauma related to handling."
- 41 buffalo randomly slaughtered for culture analysis and "all animals were culture negative for *B. abortus*."
- 1 female was euthanized due to "trauma at the handling facilities."
- 1 female died "unknown causes."

+ Unknown number of calves born in captivity 2010.

+ Unknown number of calves born in captivity 2011.

1 female from the 1st cohort has not calved, a requirement of the quarantine. "She may be released from quarantine after being spayed to ensure she does not get pregnant outside of the quarantine procedures."

Buffalo in quarantine at Slip N Slide: 68.

As you know, the Freedom of Information Act (FOIA) provides that if portions of a document are exempt from release, the remainder must be segregated and disclosed. We expect to receive all non-exempt portions of the documents that we have requested, and ask that you justify any deletions by reference to specific exemptions allowed under the FOI Act. The Buffalo Field Campaign reserves the right to appeal a decision to withhold any materials.

We hereby request a fee waiver for all search and duplication fees under the FOIA regulations [5 U.S.C. Sec. 552 (a) (4) (A) and 36 CFR 2.19(c) (1)]. The information requested will benefit the citizens of the United States and is for the purpose of public education and to encourage public debate on important policy issues. The requested information will be made available to the public through Buffalo Field Campaign's central office and/or our website.

Information available through the office and website is used in press conferences and releases, television and radio interviews, and regional and national publications, and reaches a significant number of individuals nationwide, including through the following news sources: New York Times, Los Angeles Times, Washington Post, CNN, CBS, ABC, NBC, Headline News, London Times, UK Guardian, Japanese and German TV, National Geographic, PBS, Associated Press (nationally syndicated), Reuters (internationally syndicated), Planet Green Discovery Channel, Examiner, Indian Country Today, News from Indian Country, Bozeman Daily Chronicle, Helena Independent Record, Billings Gazette, Missoulian, Great Falls Tribune, West Yellowstone News,

Livingston Enterprise, Montana Pioneer, Montana Standard, Flathead Beacon, Missoula Independent, Big Sky Weekly, Montana Public Radio, Pacifica Radio Stations, WBAI First Voices Indigenous Radio, KBZK-TV Bozeman, KXLF-TV Butte, ABC Montana, NBC Montana, CBS Montana, KGNU Colorado, Fox News Channel 8 Cleveland, Montana News Casper Star Tribune, Planet Jackson Hole, Jackson Hole News & Guide, Jackson Hole Weekly, Island Park News, Salt Lake Tribune, Powell Tribune, Ag Information Network, Idaho Statesman, Huffington Post, Word Press, New West, Yahoo! News, AlterNet, Mother Jones, Prairie Star, The Republic, Environmental News Service, Earth First! Journal, Mother Nature Network, CounterPunch, Animal People, Independent Media, multiple blogs and online news resources.

The language of the FOIA clearly indicates that Congress intended fees not to be a barrier to private individuals or public interest organizations seeking access to government records. In addition, the legislative history of the FOIA fee waiver language indicates that Congress intended a liberal interpretation of the phrase "primarily benefiting the public." This suggests that all fees are to be waived whenever the release of information contributes to public debate on important policy issues. This has been affirmed by the US Court of Appeals for the District of Columbia, in *Better Government Association v. Department of State*, 780 F. 2d 86 (D.C. Cir. 1986). In that case, the court found that under the FOIA, Congress had explicitly recognized the need for non-profit organizations to have free access to government documents and those government agencies cannot impair this free access by charging duplication or search for FOIA information requests. *Id.* at 89.

I appreciate your help and prompt response. Thank you for your time.

Sincerely,

/s/
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
<http://www.buffalofieldcampaign.org>



From: [Rhyan, Jack C \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Fagerstone, Kathleen A \(APHIS\)](#); [Miller, Lowell A \(APHIS\)](#)
Subject: FW: GonaCon Conference Call
Date: Tuesday, June 21, 2011 10:07:34 AM

FYI

From: Stephens, Stephanie H (APHIS)
Sent: Monday, June 20, 2011 2:21 PM
To: Donch, Debra A (APHIS); Willard, Tracy A (APHIS); Edmundson, Jack P (APHIS); Rhyan, Jack C (APHIS); Gutierrez, Vicki L (APHIS); Nasr, Ann M (APHIS)
Subject: GonaCon Conference Call

Hi Everyone-

Based on responses about availability, I've reserved a conference call line tomorrow for us to discuss the questions below on the GonaCon bison protocol. Here are the meeting details:

Date: Tuesday, June 21, 2011
Time: 3:30 ET (1:30 MT)
Phone: (b) (6) [REDACTED]
Code: (b) (6) [REDACTED]

Jack R., I can pass this information along to Kathy Fagerstone if you think it would be good to have her participation on the call as well to weigh in on APHIS Wildlife Services issues related to this project.

Thanks,

Stephanie

Stephanie Stephens
USDA APHIS PPD
Environmental and Risk Analysis Services
Headquarters: 4700 River Road, Unit 149, Riverdale, MD 20737
Utah Office phone/fax: (435) 658-5134

From: Edmundson, Jack P (APHIS)
Sent: Friday, June 10, 2011 12:59 PM
To: Rhyan, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: Some Q's on the GonaCon protocol and request for conf call

Hi, Jack. We pulled the Bison Team together the other day to begin work in earnest on the GonaCon EA. The first thing we did was go through the protocol with a fine-toothed comb to be sure we understood exactly what we are planning to do. Based on some things we have seen from BFC we suspect that they will be all over the study and watching like a hawk. As I understand it, the propocol you sent us is the final one that has been approved by NPS and a permit has been issued

based on it. (In other words, APHIS shouldn't change anything in it because it would be a major paperwork hassle.) With that as background, we do have a few comments/questions about the protocol:

- How come we need a YNP permit to do work outside of the Park? And what exactly does the permit cover and not cover?
- For NEPA purposes, is the lead agency APHIS or APHIS-VS? Will NPS (or NPS and APHIS-WS) officially be a cooperator in the EA? If NPS is an official cooperator, it could add additional review/approval time because NPS would have to be involved. Does NPS expect to be a NEPA Cooperator?
- What is the relationship of the study to FIFRA Registration?
- What are the roles of WS and NPS? Will they actually help in the field? Analyze info? Review/comment on things?
- The study says it starts on June 1, 2011, presumably because we collected animals after that? From a NEPA standpoint, we would prefer to have it start in 2012 when we begin to inject animals. We have already said that NEPA did not need to be done to collect animals for research. And, if we say it has already started, then technically NEPA should already be completed. (Also, for a 7 year study, it should end in 2019, not 2017.)
- Is Cammie Johnson our statistician? Should we list her in the investigators?
- The 3rd Objective does not seem to have a hypothesis associated with it. Also, the only thing in the Methods/Procedures section that could relate is the paragraph talking about what is to happen if there is an abortion in the field. It is not tied together very clearly (at least not enough for us to explain it to the public, as we must do in the EA).
- In several places we talk about marking animals, but it is not real clear how. For instance on p.4 #8 we mention collars, but elsewhere we talk about ear tags and microchips. We will need to talk about which methods we use and when.
- There is some confusion in our minds about the months when things happen. For instance, on page 5 we identify a time period when bulls will be separated from cows as outside the breeding season (from Oct to July), and the abortion/calving season from Feb to Aug. These dates will allow bulls to be with cows in August, when they could be exposed to abortions/birth-related shedding.
- We were confused by the statistics section and will probably need to be walked through that so that we can understand what we are measuring and what it means.
- There is also some confusion about when we can donate to food banks, when incineration will be used, when chemicals will be used for immobilization and/or euthanasia.

There are additional small points we would want to just talk with you about to get them straight in our minds or to ask your advice as to how to best present them in an EA. Can we organize a conference call with you to talk some of these things out? Since I am getting ready to retire, I'll be phasing out of the bison business (one of my regrets at retiring) and Stephanie Stephens will be taking my place. Since she (and Vicki) will be leading the NEPA effort, she will be getting in contact with you to set up the conference call, but we wanted you to have at least a partial list of the things we have been thinking about.

Jack E

From: [Nol, Pauline - APHIS](#)
To: [Orahood, Darcy S - APHIS \(Darcy.Orahood@aphis.usda.gov\)](#)
Subject: FW: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 2:06:00 PM

Hey Darcy,

Do you remember if you put this prep from 2 years ago through the emulsion machine or if you did it by hand?

We are aiming for the week of May 7th but still need to powwow about the prep before we place our official order😊

Thanks!

Pauline

From: Orahood, Darcy S - APHIS
Sent: Friday, March 30, 2012 8:34 AM
To: Nol, Pauline - APHIS; Miller, Lowell A - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: GonaCon study bison-Montana

I'll manufacture within the next 2 weeks and get with one of you to transfer the material once it is loaded in syringes, by Friday April 13th. Do you need any sham doses?

Thanks,

Darcy Orahood
Biological Science Technician
USDA National Wildlife Research Center
4101 LaPorte Ave
Fort Collins, CO 80521
(970) 266-6061

From: Nol, Pauline - APHIS
Sent: Wednesday, March 28, 2012 1:48 PM
To: Miller, Lowell A - APHIS; Orahood, Darcy S - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: GonaCon study bison-Montana



Hi Darcy and Lowell,

Our target date of vaccinating the bison up in Montana is April 15! Time has been flying for sure!

We would like 20 doses of 3000ug/syringe (3 ml) GonaCon by that time. Will that work with your

schedules?

Thanks and let us know of any foreseeable problems or questions.

Pauline

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-Western Region

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 3:42:00 PM

The last batch of Gonacon was made in the microfluidizer.

Do we want to use the same prep as the first group or use the hand emulsion prep?

From: Orahood, Darcy S - APHIS
Sent: Tuesday, April 08, 2014 2:35 PM
To: Nol, Pauline - APHIS
Subject: RE: GonaCon study bison-Montana

Hi Pauline,

Yes, according to my manufacturing and distribution records, this was the standard EPA-registered formulation (deer/horses) and it was indeed processed through the microfluidizer.

I'm waiting on some reagents to come in so I won't manufacture any vaccine until next week or the following week. I'll keep an eye out for your "official order" e-mail whenever you've decided on final numbers, volume, etc. 😊

Thanks,

DSO

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 2:07 PM
To: Orahood, Darcy S - APHIS
Subject: FW: GonaCon study bison-Montana

Hey Darcy,

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Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: GonaCon study bison-Montana

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Thanks,

Darcy Orahood
Biological Science Technician
USDA National Wildlife Research Center
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Fort Collins, CO 80521
(970) 266-6061

From: Nol, Pauline - APHIS
Sent: Wednesday, March 28, 2012 1:48 PM
To: Miller, Lowell A - APHIS; Orahood, Darcy S - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: GonaCon study bison-Montana



Hi Darcy and Lowell,

Our target date of vaccinating the bison up in Montana is April 15! Time has been flying for sure!

We would like 20 doses of 3000ug/syringe (3 ml) GonaCon by that time. Will that work with your schedules?

Thanks and let us know of any foreseeable problems or questions.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: FW: Green Ranch Quarantine Bison
Date: Tuesday, November 06, 2012 4:21:00 PM
Attachments: [Jorge Silva-Bañuelos.vcf](#)

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, October 31, 2012 1:44 PM
To: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: FW: Green Ranch Quarantine Bison

I spoke with Jorge this morning. Jorge was with the DOI group that we gave a tour of the GonaCon leases to on August 13 , so he has an appreciation of the facilities we are using for the study. I discussed with him four things initially:

- a) We have or have had recently brucellosis positive animals at all three leases.
- b) We would have to think carefully about the placement of Green Ranch animals to make the risk of exposure as close to zero as possible
- c) The public perception will be that Green Ranch animal are being housed in with or next to positive animals
- d) We want to have room for the 2nd cohort of GonaCon animals when they come from the trap this winter.....2-3 months could mean 4-6 months especially with a new Governor.

Jorge said the alternative he is pushing is to keep them at the Green Ranch until the 2013 roundup, but they were exploring the other options.

Ryan

From: Silva-Banuelos, Jorge G [mailto:Jorge_Silva-banuelos@ios.doi.gov]
Sent: Wednesday, October 31, 2012 12:42 PM
To: Clarke, Patrick R. - APHIS
Subject: Green Ranch Quarantine Bison

Ryan –

It was a pleasure speaking with you today. As we discussed on the phone, the Green Ranch is planning to hold a roundup of the Yellowstone-origin quarantine bison in early December. The State of Montana would like to transfer 27 of those bison (calves of the year and possibly some yearlings) to the National Bison Range. During this roundup, samples will be collected to conduct genetic and health testing of the animals to determine which bison would be suitable for transfer to NBR and what level of NEPA compliance would be necessary. However, because the Green Ranch is only planning to hold one roundup this year, the 27 bison would also need to be loaded up onto a truck and moved off the Green Ranch or else they would need to stay there until the fall 2013 roundup. Because the FWS cannot move the quarantine bison to NBR until after the genetic results are back, this scenario would require relocating the 27 bison to another location for an interim period. While the State of Montana is checking with the Department of Livestock to determine the availability of a suitable location for this purpose, I also wanted to check with you to determine the availability and suitability of using a pasture at Corwin Springs (or another APHIS-managed facility) to hold these 27 bison for an interim period - likely somewhere between 2-3 months as the genetic results are expected to come back 6-8 weeks after the samples are sent to the lab. Obviously, a critical factor in determining the location's suitability is whether the bison can be placed there without compromising their brucellosis-negative status having gone through the QFS. But I defer to you and your colleagues to identify other factors that help to evaluate the site's suitability.

I sincerely appreciate your willingness to look at this option further. If you have any other questions, please do not hesitate to contact me.

Thanks again,

Jorge Silva-Bañuelos | Special Assistant | Office of the Assistant Secretary for Fish & Wildlife and Parks

Department of the Interior | 1849 C Street NW | Room 3148 | Washington, DC 20240 | 📞 202.208.6211 (direct)

jorge@ios.doi.gov

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: FW: Green Ranch Quarantine Bison
Date: Tuesday, November 06, 2012 4:21:00 PM

From: Frey, Rebecca K - APHIS

Sent: Wednesday, October 31, 2012 4:18 PM

To: Clarke, Patrick R. - APHIS; Herriott, Donald E - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: RE: Green Ranch Quarantine Bison

So, I feel there is no possible way we can take those animals back. There will be brucellosis positive animals at every place, could be as early as Feb. if bison come out of the Park. Two of the locations have animals that WILL BE aborting, we can not have those animals in any kind of proximity in case birds etc. move fetal material around. They are silly to want to take them any closer to YNP in general.....they need to get away from here, not closer to the Hot Zone! I hope they don't try to push us from the top.....

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: Clarke, Patrick R. - APHIS

Sent: Wednesday, October 31, 2012 1:44 PM

To: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: FW: Green Ranch Quarantine Bison

I spoke with Jorge this morning. Jorge was with the DOI group that we gave a tour of the GonaCon leases to on August 13, so he has an appreciation of the facilities we are using for the study. I discussed with him four things initially:

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Jorge said the alternative he is pushing is to keep them at the Green Ranch until the 2013 roundup, but they were exploring the other options.

Ryan

From: Silva-Banuelos, Jorge G [mailto:Jorge_Silva-banuelos@ios.doi.gov]

Sent: Wednesday, October 31, 2012 12:42 PM

To: Clarke, Patrick R. - APHIS

Subject: Green Ranch Quarantine Bison

Ryan –

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to the National Bison Range. During this roundup, samples will be collected to conduct genetic and health testing of the animals to determine which bison would be suitable for transfer to NBR and what level of NEPA compliance would be necessary. However, because the Green Ranch is only planning to hold one roundup this year, the 27 bison would also need to be loaded up onto a truck and moved off the Green Ranch or else they would need to stay there until the fall 2013 roundup. Because the FWS cannot move the quarantine bison to NBR until after the genetic results are back, this scenario would require relocating the 27 bison to another location for an interim period. While the State of Montana is checking with the Department of Livestock to determine the availability of a suitable location for this purpose, I also wanted to check with you to determine the availability and suitability of using a pasture at Corwin Springs (or another APHIS-managed facility) to hold these 27 bison for an interim period - likely somewhere between 2-3 months as the genetic results are expected to come back 6-8 weeks after the samples are sent to the lab. Obviously, a critical factor in determining the location's suitability is whether the bison can be placed there without compromising their brucellosis-negative status having gone through the QFS. But I defer to you and your colleagues to identify other factors that help to evaluate the site's suitability. I sincerely appreciate your willingness to look at this option further. If you have any other questions, please do not hesitate to contact me.

Thanks again,

Jorge Silva-Bañuelos | Special Assistant | Office of the Assistant Secretary for Fish & Wildlife and Parks

Department of the Interior | 1849 C Street NW | Room 3148 | Washington, DC 20240 | ☎ 202.208.6211 (direct)
jorge@ios.doi.gov

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: HC for Bison bulls
Date: Thursday, October 08, 2015 12:59:10 PM
Attachments: [MT HC 81-454415 Bison to NWRC.pdf](#)

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, August 20, 2014 3:22 PM
To: keith.roehr@state.co.us
Cc: Rhyon, Jack C - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: HC for Bison bulls

Keith

Here is a scanned copy of the HC I wrote today for the 7 bulls that will be transported from Corwin Springs to NWRC on Friday (22nd). If you could issue a permit number I will note it on the HC in the proper box

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

CERTIFICATE OF VETERINARY INSPECTION

81 - 454415

TO ACCOMPANY SHIPMENT

CONSIGNOR NAME AND ADDRESS APHIS, VS, GonaCon Corwin Springs, MT		CONSIGNEE NAME AND ADDRESS APHIS, VS, NWRC 4101 LaPorte Ave		PERMIT NO.	DATE ISSUED 20 AUG 14
ORIGIN ADDRESS (IF DIFFERENT THAN ABOVE)		DESTINATION ADDRESS (IF DIFFERENT THAN ABOVE) Ft Collins, Co. 80521		BRAND INSP. NO.	DATE INSPD. 20 AUG 14
PURPOSE OF MOVEMENT: <input type="checkbox"/> BREEDING <input type="checkbox"/> SLAUGHTER <input type="checkbox"/> FEEDING <input checked="" type="checkbox"/> EXHIBITION, ETC. Research		AREA OF ORIGIN STATUS: <input type="checkbox"/> TB MODIFIED ACCREDIT <input type="checkbox"/> TB FREE <input type="checkbox"/> BRUCELLOSIS FREE <input type="checkbox"/> PRV STAGE V <input checked="" type="checkbox"/> OTHER: DSA		REPLICA CERTIFICATE YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>	
SPECIES: <input type="checkbox"/> CATTLE <input type="checkbox"/> HORSES <input type="checkbox"/> SHEEP <input type="checkbox"/> SWINE <input type="checkbox"/> POULTRY <input checked="" type="checkbox"/> OTHER: Bison		CARRIER: <input checked="" type="checkbox"/> TRUCK <input type="checkbox"/> OTHER:		VACCINATION OR TREATMENT FOR (EXCEPT BRUCELLOSIS) PRODUCT: DATE:	
ORIGIN OF SHIPMENT: A) County: Park B) Market:		NAME & ADDRESS: APHIS VS 4101 LaPorte Ave Ft. Collins, CO 80521		RECORD NEGATIVE TEST RESULTS LAB:	

EAR TAG NO. TATTOO OR OTHER PERMANENT IDENTIFICATION	LINE NO.	REGISTRATION NAME AND NUMBER OR DESCRIPTION	VACCINATION TATTOO SYMBOL OR DATE	AGE	SEX	BREED	Disease: Type of Test: DATE	Disease: Type of Test: DATE
81 AJW 3760	1	Red 65	N/A	1y	M	Bison		
81 AJW 3757	2	Red 69		2y				
81 AJW 3774	3	Red 61		1y				
YNP 930781	4	Red 63		2y				
YNP 930786	5	Red 66		2y				
YNP 930797	6	Red 59		2y				
YNP 930798	7	Red 62		2y				
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							

VETERINARY CERTIFICATION:

I certify as an Accredited Veterinarian that the above described animals have been inspected by me and that they are not showing signs of infectious, contagious, or communicable disease (except as noted). The vaccinations and results of tests are as indicated on the certificate. To the best of my knowledge the animals shown on this certificate meet State of Destination and Federal Interstate requirements. No warranty is made or implied.

Date: **20 AUG 14** Accredited Veterinarian Signature: **Phy Clarke**

Printed Last Name: **CLARKE**

Address: **(b) (6)**

License #: **(b) (6)**
Tel. No.: **(b) (6)**

OWNER/AGENT STATEMENT (where applicable)

"The animals in this shipment are those certified to and listed on this certificate."

Signature of Owner/Agent: **(b) (6)**

Address: **(b) (6)**

Date: **20 AUG 14**

From: [O'Hare, Jeanette R \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#)
Subject: FW: Meeting to discuss the Bison Study
Date: Tuesday, August 16, 2011 1:40:10 PM

Here is the e-mail with a couple comments including the water.

From: O'Hare, Jeanette R (APHIS)
Sent: Thursday, June 23, 2011 12:00 PM
To: Nol, Pauline (APHIS)
Subject: RE: Meeting to discuss the Bison Study

Pauline,

I checked the GonaCon ingredients in the protocol. The only thing you might change is the water. It is really just "distilled water".

But I did not see anything related to "efficacy" per say. 1) I didn't see anything about GnRH titers. Is it in a later version or amendment? 2) Calving rates/pregnancy are necessary for your other study objectives, but not specifically mentioned in relation to GonaCon efficacy. If you have to write an amendment, maybe it could be related to the efficacy issue. Just a thought.

Let me know if you need anything.

Jeanette

From: Nol, Pauline (APHIS)
Sent: Friday, June 03, 2011 3:24 PM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study

Here is the latest draft of QA1858. Please check on the regulatory requirements and corresponding appendices. I'll attach the approved ACUC once we are ready to submit. And I'll touch base with Cathy Bens before we do as well. Where I have comment balloons I was not sure what to fill in.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126

Mobile: (b) (6)

From: Eisemann, John D (APHIS)

Sent: Friday, June 03, 2011 10:46 AM

To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)

Subject: Meeting to discuss the Bison Study

Jack and Kathy just set up a meeting at 2:00 pm (MT) to discuss the bison study. There are some important registration considerations that need to be discussed before the study planning goes too far. Hope you can make it. It will be in the conference room by my office. Stephanie, I will call you if you are available.

John D. Eisemann

National Wildlife Research Center

4101 Laporte Avenue

Fort Collins, CO 80526

T: 970-266-6158

F: 970-266-6157

John.D.Eisemann@aphis.usda.gov

From: [Rhyen, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: my blurbs on our collaborative work with ARS
Date: Wednesday, February 01, 2012 9:07:59 AM
Attachments: [ARS-APHIScollaborative projects.docx](#)

From: Herriott, Donald E - APHIS
Sent: Wednesday, February 01, 2012 9:06 AM
To: Rhyen, Jack C - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

From: Rhyen, Jack C - APHIS
Sent: Monday, January 23, 2012 1:29 PM
To: Herriott, Donald E - APHIS
Subject: RE: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

Don,

Please see what you think about this.

Thanks,

Jack

From: Herriott, Donald E - APHIS
Sent: Monday, January 23, 2012 9:12 AM
To: Rhyen, Jack C - APHIS; Clarke, Patrick R. - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
Can you send me a "gonacon study summary page" asap?
thx

From: Davidson, Mark L - APHIS
Sent: Monday, January 23, 2012 8:58 AM
To: Herriott, Donald E - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
See below, too quick with send button

From: Davidson, Mark L - APHIS
Sent: Monday, January 23, 2012 8:58 AM
To: Burke L Healey
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
Burke, Can you check with Kevin and see if we need any new support from ARS on CFTEP or there are particular items we want to highlight for continued support?
Don, Anything GYA?
Will need the info, sometime this week.

From: Dick, Jere L - APHIS
Sent: Friday, January 20, 2012 1:12 PM
To: Anelli, Joseph F - APHIS; McCluskey, Brian J - APHIS; Shere, Jack A - APHIS; Davidson, Mark L - APHIS
Cc: Fisher, Sharon S - APHIS; Christensen, Laura C - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
Do any of you have any ARS specific items that need to be discussed at the Senior Management level?

From: Fisher, Sharon S - APHIS
Sent: Friday, January 20, 2012 3:10 PM
To: APHIS-VS Executive Team
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
Hi all – We are need of items for APHIS-ARS meeting. Seems like there should be something what with all the NBAF and pre-harvest activity of late. Please let me know by TUESDAY next week.

From: Lewandowski, Laura P - APHIS

Sent: Friday, January 20, 2012 8:47 AM

To: Christensen, Laura C - APHIS; Cooper, Julie F - APHIS; Fisher, Sharon S - APHIS; Griffith, Carol A - APHIS; Hancock, Jason M - APHIS; Joyce, Carrie E - APHIS; Lohs, Christina L - APHIS; Mahoney, Bridget C - APHIS; Myers, Christina J - APHIS; Nesbitt, Thomas C - APHIS; Shelor, Steven L - APHIS; Tuck, James M - APHIS; White, Robin L - APHIS

Cc: Barsi, Janel L - APHIS

Subject: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

The next bimonthly APHIS/ARS meeting is scheduled for Friday February 3, 2012 from 1:00 – 2:00 in conference room 305-E. In advance of that meeting, please send me any of your suggested agenda items by cob Thursday January 26. We will schedule a pre-meeting for sometime during the week of January 23 so I'll be in touch on that.

Thanks!

Laura Porcella Lewandowski

Acting Deputy Chief of Staff

Animal and Plant Health Inspection Service

1400 Independence Avenue, SW

Jamie L. Whitten Building, Room 314-E

Washington, D.C. 20250

202- 690-2288 (Monday, Wednesday, Friday)

301-436-3185 (Tuesday and Thursday)

APHIS VS and ARS are collaboratively engaged in three areas of research involving wildlife:

1. Investigations into the use of BCG as an oral vaccine to prevent bovine tuberculosis in white-tailed deer and feral swine.

This project involves several studies of which some are completed and some are in progress. The end goal is to conduct field trials in Michigan white-tailed deer and in feral swine on the Island of Molokai. If field trials are successful, the vaccine would be available for licensing and use as a disease management and eradication tool. Past studies between investigators at Fort Collins and NADC, Ames, have focused on vaccine efficacy and interspecies transmission. Current studies are evaluating the persistence of the vaccine strain in deer and swine.

2. Investigations into the use of volatile organic compounds as a diagnostic tool for the detection of brucellosis, tuberculosis, and paratuberculosis in wild and domestic animals.

Preliminary studies by APHIS investigators have shown that the breath of *Mycobacterium bovis*-infected cattle contain unique volatile organic compounds (VOCs) and VOC profiles detectable by gas chromatography-mass spectrophotometry (GC-MS) and two types of electronic noses (e-noses). Current and planned studies by APHIS personnel, in collaboration with investigators at NADC, are evaluating the breath of cattle, deer, and swine infected with *M. bovis*, *Brucella abortus*, and *Mycobacterium paratuberculosis* for unique VOCs and VOC profiles. If successful, this technology will have utility in the remote detection of diseases in wildlife and some domestic animal industries.

3. Investigations into the use of GonaCon®, an immunocontraceptive vaccine, to eliminate shedding of *B. abortus* from infected bison.

Preliminary studies by APHIS/VS, and APHIS/WS investigators has shown a single injection of GonaCon® produces approximately 3 years of infertility in treated bison. A study, to begin this Spring, will evaluate shedding of *B. abortus*-treated and untreated bison held in quarantine. It will also provide more data on the duration of infertility in bison treated with this product and will provide further information on the pathogenesis of brucellosis in bison. Portions of the project will involve collaboration with investigators at NADC. If successful, this study could lead to licensing of the use of this product in bison and provide a non-lethal tool to eliminate brucellosis from an infected bison population.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#14-021012,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Monday, January 05, 2015 4:37:11 PM
Attachments: [14-021012_DBL-BRUC_ALT_01-05-2015-05-31-11-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Monday, January 05, 2015 3:33 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: NVSL Report - Accession#14-021012,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-021012

Date Received: 07/03/2014 10:44:05 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA, APHIS, VS-GonaCon Study
Corwin Springs, MT**Accession Number:** 14-021012**Animal Location**

Park County MT

Date Collected:**Date Received:** 07/03/2014**Submitter - 1961**

DR Patrick Ryan Clarke

Date Completed:**Collected By:** Frey Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Purpose:

General Diagnostic

Belgrade, MT 59714

FAX #: 406-866-5162

Referral Number:**This is not a billable case.**

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** Red11 **Animal ID:** Red11 **Brucella Case Number:** B14-0472 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3R20 **Animal ID:** 3R20 **Brucella Case Number:** B14-0473 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B14-0474 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Gr03 **Animal ID:** Gr03 **Brucella Case Number:** B14-0475 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B14-0476 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red01 **Animal ID:** Red01 **Brucella Case Number:** B14-0477 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B14-0478 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B14-0479 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red28 **Animal ID:** Red28 **Brucella Case Number:** B14-0480 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red04 **Animal ID:** Red04 **Brucella Case Number:** B14-0481 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red14 **Animal ID:** Red14 **Brucella Case Number:** B14-0482 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B14-0483 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B14-0484 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 4G10 **Animal ID:** 4G10 **Brucella Case Number:** B14-0485 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Skin / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B14-0486 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, January 07, 2015 12:32:42 PM
Attachments: [14-029714_DBL-BRUC_ALT_01-07-2015-01-22-41-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, January 07, 2015 12:26 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 14-029714

Date Received: 09/16/2014 09:30:41 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Embryo Transfer Study
Ft. Collins, CO

Accession Number: 14-029714

Animal Location

Park County MT

Date Collected: 08/21/2014

Date Received: 09/16/2014

Submitter - 2649

DR Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140

Date Completed: 01/07/2015

Collected By: Jack Rhyan

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 49 **Animal ID:** 49 **Brucella Case Number:** B14-0564 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Placenta / Placenta

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Swab / Swab, Placenta

Brucella Isolation Result

Contaminated

Milk / Milk- Left Front

Brucella Isolation Result

No Isolation Made

Milk / Milk- Left Rear

Brucella Isolation Result

No Isolation Made

Milk / Milk- Right Front

Brucella Isolation Result

No Isolation Made

Milk / Milk- Right Rear

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Placenta and Placenta swab samples were very heavily contaminated. Both were overgrown with swarming bacteria, so the amount of Brucella could not be estimated.

Sample: 49 Calf **Animal ID:** 49 Calf **Brucella Case Number:** B14-0565 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Lymph Node Pool / Lymph Node- Not Identified

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Lymph Node / Lymph Node- Throacic Region

Brucella Isolation Result

Suspect Isolated

Lymph Node / Lymph Node- Abdominal Region

Brucella Isolation Result

Suspect Isolated

Spleen / Spleen

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Lung / Lung

Brucella Isolation Result

Suspect Isolated

Liver / Liver

Brucella Isolation Result

Suspect Isolated

Estimated amount of Brucella on inital isolation:**LN-NI: Confluent lawn of *Brucella*****Thoracic LN: Confluent lawn of *Brucella*****Abdominal LN: >300 colonies****Spleen: ~250 colonies****Lung: Confluent lawn of *Brucella*****Liver: ~250 colonies****Results authorized by:**Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**Help Us Help You**

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#14-041526,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Monday, January 05, 2015 4:38:22 PM
Attachments: [14-041526_DBL-BRUC_ALT_01-05-2015-05-31-13-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Monday, January 05, 2015 3:33 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: NVSL Report - Accession#14-041526,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-041526

Date Received: 12/17/2014 11:01:24 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS-GonaCon Study

Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

DR Patrick Ryan Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Accession Number:**14-041526****Date Collected:**

07/15/2014

Date Received:

12/17/2014

Date Completed:

01/05/2015

Collected By:

R. Frey, et al

Purpose:

General Diagnostic

Referral Number:**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Grn21 **Animal ID:** Grn21 **Brucella Case Number:** B14-0701 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Grn24 **Animal ID:** Grn24 **Brucella Case Number:** B14-0702 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Grn25 **Animal ID:** Grn25 **Brucella Case Number:** B14-0703 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#15-014777,Purpose:GEN_DIAG,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov
Date: Tuesday, July 07, 2015 1:11:48 PM
Attachments: [15-014777_DBL-BRUC_ALT_07-07-2015-11-56-48-AM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Tuesday, July 07, 2015 10:57 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: NVSL Report - Accession#15-014777,Purpose:GEN_DIAG,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-014777

Date Received: 05/07/2015 09:46:56 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA, APHIS, VS
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number:

15-014777

Date Collected:

Date Received:

05/07/2015

Date Completed:

Collected By:

07/06/2015

Frey Clarke

Purpose:

General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 5G01 **Animal ID:** 5G01 **Brucella Case Number:** B15-0126 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G03 **Animal ID:** 5G03 **Brucella Case Number:** B15-0127 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G04 **Animal ID:** 5G04 **Brucella Case Number:** B15-0128 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G06 **Animal ID:** 5G06 **Brucella Case Number:** B15-0129 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G08 **Animal ID:** 5G08 **Brucella Case Number:** B15-0130 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G09 **Animal ID:** 5G09 **Brucella Case Number:** B15-0131 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G13 **Animal ID:** 5G13 **Brucella Case Number:** B15-0132 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5G15 **Animal ID:** 5G15 **Brucella Case Number:** B15-0133 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R02 **Animal ID:** 5R02 **Brucella Case Number:** B15-0134 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R03 **Animal ID:** 5R03 **Brucella Case Number:** B15-0135 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R13 **Animal ID:** 5R13 **Brucella Case Number:** B15-0136 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R14 **Animal ID:** 5R14 **Brucella Case Number:** B15-0137 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R18 **Animal ID:** 5R18 **Brucella Case Number:** B15-0138 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 5R26 **Animal ID:** 5R26 **Brucella Case Number:** B15-0139 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G01 **Animal ID:** Green 01 **Brucella Case Number:** B15-0140 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G02 **Animal ID:** Green 02 **Brucella Case Number:** B15-0141 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G03 **Animal ID:** Green 03 **Brucella Case Number:** B15-0142 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G04 **Animal ID:** Green04 **Brucella Case Number:** B15-0143 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G06 **Animal ID:** Green06 **Brucella Case Number:** B15-0144 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G07 **Animal ID:** Green07 **Brucella Case Number:** B15-0145 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G08 **Animal ID:** Green08 **Brucella Case Number:** B15-0146 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G09 **Animal ID:** Green09 **Brucella Case Number:** B15-0147 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G13 **Animal ID:** Green13 **Brucella Case Number:** B15-0148 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G15 **Animal ID:** Green 15 **Brucella Case Number:** B15-0149 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R02 **Animal ID:** Red02 **Brucella Case Number:** B15-0150 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R03 **Animal ID:** Red 03 **Brucella Case Number:** B15-0151 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R06 **Animal ID:** Red 06 **Brucella Case Number:** B15-0152 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Tissue / Placenta

Brucella Isolation Result

No Isolation Made

Sample: R09 **Animal ID:** Red09 **Brucella Case Number:** B15-0153 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Tissue / Placenta

Brucella Isolation Result

Suspect Isolated

Sample: R13 **Animal ID:** Red13 **Brucella Case Number:** B15-0154 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R14 **Animal ID:** Red 14 **Brucella Case Number:** B15-0155 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R18 **Animal ID:** Red 18 **Brucella Case Number:** B15-0156 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Discharge, Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R26 Animal ID: Red26 Brucella Case Number: B15-0157 Specimen Type: Multiple - Specify in Sample Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal	
Brucella Isolation Result	No Isolation Made
Milk / Milk	
Brucella Isolation Result	Contaminated
Feces / Feces	
Brucella Isolation Result	Contaminated
Blood / Blood	
Brucella Isolation Result	No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#15-017999,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov
Date: Tuesday, July 14, 2015 3:07:04 PM
Attachments: [15-017999_DBL-SERO_FI2_06-26-2015-09-22-21-AM.pdf](#)

-----Original Message-----

From: Rhyan, Jack C - APHIS
Sent: Tuesday, June 30, 2015 9:48 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: NVSL Report - Accession#15-017999,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Friday, June 26, 2015 8:29 AM
To: Rhyan, Jack C - APHIS
Subject: NVSL Report - Accession#15-017999,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 15-017999

Date Received: 06/04/2015 08:00:23 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Bison Quarantine-G. Study
Corwin Springs, MT

Accession Number: 15-019452

Animal Location

Park County MT

Date Collected:

Date Received: 06/16/2015

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 07/16/2015

Collected By: R. Frey

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B15-0238 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B15-0239 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G08 **Animal ID:** 4G08 **Brucella Case Number:** B15-0240 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G17 **Animal ID:** 4G17 **Brucella Case Number:** B15-0241 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R06 **Animal ID:** 4R06 **Brucella Case Number:** B15-0242 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B15-0243 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R22 **Animal ID:** 4R22 **Brucella Case Number:** B15-0244 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R01 **Animal ID:** R01 **Brucella Case Number:** B15-0245 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R04 **Animal ID:** R04 **Brucella Case Number:** B15-0246 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R05 Animal ID: R05 Brucella Case Number: B15-0247 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R11 Animal ID: R11 Brucella Case Number: B15-0248 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R17 Animal ID: R17 Brucella Case Number: B15-0249 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R19 Animal ID: R19 Brucella Case Number: B15-0250 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R25 Animal ID: R25 Brucella Case Number: B15-0251 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R27 Animal ID: R27 Brucella Case Number: B15-0252 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R28 Animal ID: R28 Brucella Case Number: B15-0253 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R29 Animal ID: R29 Brucella Case Number: B15-0254 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R31 Animal ID: R31 Brucella Case Number: B15-0255 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Thursday, July 16, 2015 2:00:28 PM
Attachments: [15-019452_DBL-BRUC_ALT_07-16-2015-12-06-38-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 11:47 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, July 16, 2015 11:36 AM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-019452

Date Received: 06/16/2015 11:49:42 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Bison Quarantine-G. Study
Corwin Springs, MT

Accession Number: 15-019452

Animal Location

Park County MT

Date Collected:

Date Received: 06/16/2015

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 07/16/2015

Collected By: R. Frey

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B15-0238 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B15-0239 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G08 **Animal ID:** 4G08 **Brucella Case Number:** B15-0240 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G17 **Animal ID:** 4G17 **Brucella Case Number:** B15-0241 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R06 **Animal ID:** 4R06 **Brucella Case Number:** B15-0242 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B15-0243 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R22 **Animal ID:** 4R22 **Brucella Case Number:** B15-0244 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R01 **Animal ID:** R01 **Brucella Case Number:** B15-0245 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R04 **Animal ID:** R04 **Brucella Case Number:** B15-0246 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R05 Animal ID: R05 Brucella Case Number: B15-0247 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R11 Animal ID: R11 Brucella Case Number: B15-0248 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R17 Animal ID: R17 Brucella Case Number: B15-0249 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R19 Animal ID: R19 Brucella Case Number: B15-0250 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R25 Animal ID: R25 Brucella Case Number: B15-0251 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R27 Animal ID: R27 Brucella Case Number: B15-0252 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R28 Animal ID: R28 Brucella Case Number: B15-0253 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R29 Animal ID: R29 Brucella Case Number: B15-0254 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R31 Animal ID: R31 Brucella Case Number: B15-0255 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Thursday, July 16, 2015 2:40:01 PM
Attachments: [15-019452_DBL-BRUC_ALT_07-16-2015-12-06-38-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 11:47 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, July 16, 2015 11:36 AM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-019452

Date Received: 06/16/2015 11:49:42 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Bison Quarantine-G. Study
Corwin Springs, MT

Accession Number: 15-019452

Animal Location

Park County MT

Date Collected:

Date Received: 06/16/2015

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 07/16/2015

Collected By: R. Frey

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B15-0238 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B15-0239 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G08 **Animal ID:** 4G08 **Brucella Case Number:** B15-0240 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G17 **Animal ID:** 4G17 **Brucella Case Number:** B15-0241 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R06 **Animal ID:** 4R06 **Brucella Case Number:** B15-0242 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B15-0243 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R22 **Animal ID:** 4R22 **Brucella Case Number:** B15-0244 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R01 **Animal ID:** R01 **Brucella Case Number:** B15-0245 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R04 **Animal ID:** R04 **Brucella Case Number:** B15-0246 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R05 Animal ID: R05 Brucella Case Number: B15-0247 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R11 Animal ID: R11 Brucella Case Number: B15-0248 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R17 Animal ID: R17 Brucella Case Number: B15-0249 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R19 Animal ID: R19 Brucella Case Number: B15-0250 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R25 Animal ID: R25 Brucella Case Number: B15-0251 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R27 Animal ID: R27 Brucella Case Number: B15-0252 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R28 Animal ID: R28 Brucella Case Number: B15-0253 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R29 Animal ID: R29 Brucella Case Number: B15-0254 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R31 Animal ID: R31 Brucella Case Number: B15-0255 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by:Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**Help Us Help You**

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#15-020347,Purpose:GEN_DIAG,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Thursday, July 30, 2015 3:01:23 PM
Attachments: [15-020347 DBL-BRUC ALT 07-30-2015-11-56-27-AM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, July 30, 2015 11:08 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: NVSL Report - Accession#15-020347,Purpose:GEN_DIAG,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline nol@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-020347

Date Received: 06/25/2015 10:24:26 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS
Corwin Springs, MT**Accession Number:** 15-020347**Animal Location**

Park County MT

Date Collected:**Date Received:** 06/25/2015**Submitter - 1961**

Dr. Patrick Ryan Clarke

Date Completed:**Collected By:** Clarke Frey

USDA, APHIS, VS

187 E. Tobiano Tr.

Purpose:

General Diagnostic

Belgrade, MT 59714

FAX #: 406-866-5162

Referral Number:

Phone #: 406-866-5162

This is not a billable case.**NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** R22 **Animal ID:** R22 **Brucella Case Number:** B15-0286 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 5R22 **Animal ID:** 5R22 **Brucella Case Number:** B15-0287 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G25 **Animal ID:** G25 **Brucella Case Number:** B15-0288 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 5G25 **Animal ID:** 5G25 **Brucella Case Number:** B15-0289 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R16 **Animal ID:** R16 **Brucella Case Number:** B15-0290 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

Contaminated

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 5R16 **Animal ID:** 5R16 **Brucella Case Number:** B15-0291 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#15-026723,Purpose:NVSL_INTER,Exam Req:JOHNES sent to jack.c.rhyan@aphis.usda.gov
Date: Monday, August 31, 2015 1:15:57 PM
Attachments: [15-026723_MB_FI_REPT_08-31-2015-08-18-57-AM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Monday, August 31, 2015 7:19 AM
To: Rhyan, Jack C - APHIS
Subject: NVSL Report - Accession#15-026723,Purpose:NVSL_INTER,Exam Req:JOHNES sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 15-026723

Date Received: 08/25/2015 11:01:17 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: JOHNES

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerJack Rhyan
Fort Collins, CO**Accession Number:****15-026723****Animal Location**

Larimer County CO

Date Collected:

08/20/2015

Date Received:

08/25/2015

Date Completed:

08/28/2015

Submitter - 2649Dr. Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140**Collected By:**

Rhyan

Purpose:

NVSL Internal

Referral/Retain Tag Number:**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Animal ID: 4G8 Case #: TB15-03506 Sample: 4G8 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 4R6 Case #: TB15-03507 Sample: 4R6 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 4R13 Case #: TB15-03508 Sample: 4R13 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected

Animal ID: 4R22 Case #: TB15-03509 Sample: 4R22 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 3G03 Case #: TB15-03510 Sample: 3G03 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 4G02 Case #: TB15-03511 Sample: 4G02 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 4G06 Case #: TB15-03512 Sample: 4G06 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 3R21 Case #: TB15-03513 Sample: 3R21 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = 39.0

Interpretation: Suspect - Very low levels of Mycobacterium
avium ssp. paratuberculosis DNA was detected. See below.**Animal ID: 4R16 Case #: TB15-03514 Sample: 4R16 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 4R? Case #: TB15-03515 Sample: 4R? Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected

Animal ID: G23 Case #: TB15-03516 Sample: G23 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = 38.6

Interpretation: Suspect - Very low levels of Mycobacterium avium ssp. paratuberculosis DNA was detected. See below.

Animal ID: G26 Case #: TB15-03517 Sample: G26 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp. paratuberculosis DNA was detected

Animal ID: 4R7 Case #: TB15-03518 Sample: 4R7 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp. paratuberculosis DNA was detected

Animal ID: 4R21 Case #: TB15-03519 Sample: 4R21 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp. paratuberculosis DNA was detected

Animal ID: 27 Case #: TB15-03520 Sample: 27 Specimen Type: Feces Species: Bison

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp. paratuberculosis DNA was detected

Cycle Threshold (Ct) provides an estimate of the amount of *M. avium* subsp. *paratuberculosis* (MAP) DNA in the fecal material. Generally the lower the number, the more DNA in the fecal material. MAP DNA can then be correlated to the number of organisms shed in the fecal material.

General guidelines are as follows:

<25 Ct = Very Heavy Fecal Shedder

<30 Ct = Heavy Fecal Shedder

<33 Ct = Moderate Fecal Shedder

<36 Ct = Light Fecal Shedder

<40 Ct = Suspect Fecal Shedder - DNA was detected, but at a low level. Without epidemiological information, correlation to fecal culture is not consistent.

Undetermined = No MAP DNA was detected

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#15-027129,Purpose:NVSL_INTER,Exam Req:JOHNES sent to jack.c.rhyan@aphis.usda.gov
Date: Monday, August 31, 2015 1:16:54 PM
Attachments: [15-027129_MB_FI_REPT_08-31-2015-08-18-57-AM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Monday, August 31, 2015 7:19 AM
To: Rhyan, Jack C - APHIS
Subject: NVSL Report - Accession#15-027129,Purpose:NVSL_INTER,Exam Req:JOHNES sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 15-027129

Date Received: 08/27/2015 09:28:49 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: JOHNES

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerJack Rhyan
Fort Collins, CO**Accession Number:****15-027129****Animal Location**

Larimer County CO

Date Collected:

08/25/2015

Date Received:

08/27/2015

Date Completed:

08/28/2015

Submitter - 2649Dr. Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140**Collected By:**

Rhyan

Purpose:

NVSL Internal

Referral/Retain Tag Number:**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Animal ID: 10 Case #: TB15-03536 Sample: 10 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 12 Case #: TB15-03537 Sample: 12 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected**Animal ID: 49 Case #: TB15-03538 Sample: 49 Specimen Type: Feces Species: Bison**

Johnes Direct PCR - Result

Ct = Undetermined

Interpretation = Negative - No Mycobacterium avium ssp.
paratuberculosis DNA was detected

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyen, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Tuesday, March 22, 2016 2:39:11 PM
Attachments: [16-004807_DBL-BRUC_ALT_03-22-2016-01-10-38-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, March 22, 2016 2:10 PM
To: Frey, Rebecca K - APHIS; Rhyen, Jack C - APHIS
Subject: FW: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Tuesday, March 22, 2016 12:12 PM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-004807

Date Received: 02/10/2016 10:50:05 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner *W i l d l i f e*

USDA, APHIS, VS- GonaCon Study
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number: **16-004807**

Date Collected:

Date Received: 02/10/2016

Date Completed:

Collected By: Clarke, et al

Purpose:

General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red 32 Animal ID: Red 32 Brucella Case Number: B16-0012 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 33 Animal ID: Red 33 Brucella Case Number: B16-0013 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 34 Animal ID: Red 34 Brucella Case Number: B16-0014 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 35 **Animal ID:** Red 35 **Brucella Case Number:** B16-0015 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 36 **Animal ID:** Red 36 **Brucella Case Number:** B16-0016 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 37 **Animal ID:** Red 37 **Brucella Case Number:** B16-0017 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 38 **Animal ID:** Red 38 **Brucella Case Number:** B16-0018 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 39 **Animal ID:** Red 39 **Brucella Case Number:** B16-0019 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 40 **Animal ID:** Red 40 **Brucella Case Number:** B16-0020 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 41 **Animal ID:** Red 41 **Brucella Case Number:** B16-0021 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 42 **Animal ID:** Red 42 **Brucella Case Number:** B16-0022 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 43 **Animal ID:** Red 43 **Brucella Case Number:** B16-0023 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 44 **Animal ID:** Red 44 **Brucella Case Number:** B16-0024 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 45 **Animal ID:** Red 45 **Brucella Case Number:** B16-0025 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 46 **Animal ID:** Red 46 **Brucella Case Number:** B16-0026 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 47 **Animal ID:** Red 47 **Brucella Case Number:** B16-0027 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 48 **Animal ID:** Red 48 **Brucella Case Number:** B16-0028 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 49 **Animal ID:** Red 49 **Brucella Case Number:** B16-0029 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 50 **Animal ID:** Red 50 **Brucella Case Number:** B16-0030 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 51 **Animal ID:** Red 51 **Brucella Case Number:** B16-0031 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 53 **Animal ID:** Red 53 **Brucella Case Number:** B16-0032 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 54 **Animal ID:** Red 54 **Brucella Case Number:** B16-0033 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 55 **Animal ID:** Red 55 **Brucella Case Number:** B16-0034 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 56 **Animal ID:** Red 56 **Brucella Case Number:** B16-0035 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 64 **Animal ID:** Red 64 **Brucella Case Number:** B16-0036 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 67 **Animal ID:** Red 67 **Brucella Case Number:** B16-0037 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 68 **Animal ID:** Red 68 **Brucella Case Number:** B16-0038 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 71 **Animal ID:** Red 71 **Brucella Case Number:** B16-0039 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 72 **Animal ID:** Red 72 **Brucella Case Number:** B16-0040 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 73 **Animal ID:** Red 73 **Brucella Case Number:** B16-0041 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 74 **Animal ID:** Red 74 **Brucella Case Number:** B16-0042 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 7 **Animal ID:** Green 7 **Brucella Case Number:** B16-0043 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 01 **Animal ID:** Green 01 **Brucella Case Number:** B16-0044 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

This sample was listed on the submission form as Green 10. The sample was labeled Green 01. Dr. Clarke confirmed the correct ID as Green 01.

Sample: Green 11 **Animal ID:** Green 11 **Brucella Case Number:** B16-0045 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 12 **Animal ID:** Green 12 **Brucella Case Number:** B16-0046 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 13 **Animal ID:** Green 13 **Brucella Case Number:** B16-0047 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 18 **Animal ID:** Green 18 **Brucella Case Number:** B16-0048 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 20 **Animal ID:** Green 20 **Brucella Case Number:** B16-0049 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 21 **Animal ID:** Green 21 **Brucella Case Number:** B16-0050 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 24 **Animal ID:** Green 24 **Brucella Case Number:** B16-0051 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 25 **Animal ID:** Green 25 **Brucella Case Number:** B16-0052 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 30 **Animal ID:** Green 30 **Brucella Case Number:** B16-0053 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B16-0054 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3G08 **Animal ID:** Or3G08 **Brucella Case Number:** B16-0055 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3G14 **Animal ID:** Or3G14 **Brucella Case Number:** B16-0056 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3R20 **Animal ID:** Or3R20 **Brucella Case Number:** B16-0057 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3R22 **Animal ID:** Or3R22 **Brucella Case Number:** B16-0058 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

[Help Us Help You](#)

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyen, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Tuesday, March 22, 2016 2:39:11 PM
Attachments: [16-004807_DBL-BRUC_ALT_03-22-2016-01-10-38-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, March 22, 2016 2:10 PM
To: Frey, Rebecca K - APHIS; Rhyen, Jack C - APHIS
Subject: FW: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Tuesday, March 22, 2016 12:12 PM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-004807,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-004807

Date Received: 02/10/2016 10:50:05 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner *W i l d l i f e*

USDA, APHIS, VS- GonaCon Study
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number: 16-004807

Date Collected:

Date Received: 02/10/2016

Date Completed:

Collected By: Clarke, et al

Purpose: General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red 32 **Animal ID:** Red 32 **Brucella Case Number:** B16-0012 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 33 **Animal ID:** Red 33 **Brucella Case Number:** B16-0013 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 34 **Animal ID:** Red 34 **Brucella Case Number:** B16-0014 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 35 **Animal ID:** Red 35 **Brucella Case Number:** B16-0015 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 36 **Animal ID:** Red 36 **Brucella Case Number:** B16-0016 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 37 **Animal ID:** Red 37 **Brucella Case Number:** B16-0017 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 38 **Animal ID:** Red 38 **Brucella Case Number:** B16-0018 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 39 **Animal ID:** Red 39 **Brucella Case Number:** B16-0019 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 40 **Animal ID:** Red 40 **Brucella Case Number:** B16-0020 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 41 **Animal ID:** Red 41 **Brucella Case Number:** B16-0021 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 42 **Animal ID:** Red 42 **Brucella Case Number:** B16-0022 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 43 **Animal ID:** Red 43 **Brucella Case Number:** B16-0023 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 44 **Animal ID:** Red 44 **Brucella Case Number:** B16-0024 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 45 **Animal ID:** Red 45 **Brucella Case Number:** B16-0025 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 46 **Animal ID:** Red 46 **Brucella Case Number:** B16-0026 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 47 **Animal ID:** Red 47 **Brucella Case Number:** B16-0027 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 48 **Animal ID:** Red 48 **Brucella Case Number:** B16-0028 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 49 **Animal ID:** Red 49 **Brucella Case Number:** B16-0029 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 50 **Animal ID:** Red 50 **Brucella Case Number:** B16-0030 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 51 **Animal ID:** Red 51 **Brucella Case Number:** B16-0031 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 53 **Animal ID:** Red 53 **Brucella Case Number:** B16-0032 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 54 **Animal ID:** Red 54 **Brucella Case Number:** B16-0033 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 55 **Animal ID:** Red 55 **Brucella Case Number:** B16-0034 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 56 **Animal ID:** Red 56 **Brucella Case Number:** B16-0035 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 64 **Animal ID:** Red 64 **Brucella Case Number:** B16-0036 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 67 **Animal ID:** Red 67 **Brucella Case Number:** B16-0037 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 68 **Animal ID:** Red 68 **Brucella Case Number:** B16-0038 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 71 **Animal ID:** Red 71 **Brucella Case Number:** B16-0039 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 72 **Animal ID:** Red 72 **Brucella Case Number:** B16-0040 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 73 **Animal ID:** Red 73 **Brucella Case Number:** B16-0041 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 74 **Animal ID:** Red 74 **Brucella Case Number:** B16-0042 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 7 **Animal ID:** Green 7 **Brucella Case Number:** B16-0043 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 01 **Animal ID:** Green 01 **Brucella Case Number:** B16-0044 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

This sample was listed on the submission form as Green 10. The sample was labeled Green 01. Dr. Clarke confirmed the correct ID as Green 01.

Sample: Green 11 **Animal ID:** Green 11 **Brucella Case Number:** B16-0045 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 12 **Animal ID:** Green 12 **Brucella Case Number:** B16-0046 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 13 **Animal ID:** Green 13 **Brucella Case Number:** B16-0047 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 18 **Animal ID:** Green 18 **Brucella Case Number:** B16-0048 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 20 **Animal ID:** Green 20 **Brucella Case Number:** B16-0049 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 21 **Animal ID:** Green 21 **Brucella Case Number:** B16-0050 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 24 **Animal ID:** Green 24 **Brucella Case Number:** B16-0051 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 25 **Animal ID:** Green 25 **Brucella Case Number:** B16-0052 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 30 **Animal ID:** Green 30 **Brucella Case Number:** B16-0053 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B16-0054 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3G08 **Animal ID:** Or3G08 **Brucella Case Number:** B16-0055 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3G14 **Animal ID:** Or3G14 **Brucella Case Number:** B16-0056 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3R20 **Animal ID:** Or3R20 **Brucella Case Number:** B16-0057 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Or3R22 **Animal ID:** Or3R22 **Brucella Case Number:** B16-0058 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

[Help Us Help You](#)

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Tuesday, March 22, 2016 2:38:01 PM
Attachments: [16-004824_DBL-BRUC_ALT_03-22-2016-01-10-39-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, March 22, 2016 2:10 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: FW: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Tuesday, March 22, 2016 12:12 PM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-004824

Date Received: 02/10/2016 11:06:25 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner *W i l d l i f e*

USDA, APHIS, VS- GonaCon Study

Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Dr. Patrick Ryan Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Accession Number: **16-004824****Date Collected:****Date Received:** 02/10/2016**Date Completed:** 03/22/2016**Collected By:** Clarke, Frey**Purpose:** General Diagnostic**Referral Number:****This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Green 2 **Animal ID:** Green 2 **Brucella Case Number:** B16-0059 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 3 **Animal ID:** Green 3 **Brucella Case Number:** B16-0060 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 4 **Animal ID:** Green 4 **Brucella Case Number:** B16-0061 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 6 **Animal ID:** Green 6 **Brucella Case Number:** B16-0062 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Green 8 **Animal ID:** Green 8 **Brucella Case Number:** B16-0063 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 9 **Animal ID:** Green 9 **Brucella Case Number:** B16-0064 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 14 **Animal ID:** Green 14 **Brucella Case Number:** B16-0065 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 15 **Animal ID:** Green 15 **Brucella Case Number:** B16-0066 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 17 **Animal ID:** Green 17 **Brucella Case Number:** B16-0067 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 1 **Animal ID:** Red 1 **Brucella Case Number:** B16-0068 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 2 **Animal ID:** Red 2 **Brucella Case Number:** B16-0069 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 3 **Animal ID:** Red 3 **Brucella Case Number:** B16-0070 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 4 **Animal ID:** Red 4 **Brucella Case Number:** B16-0071 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 5 **Animal ID:** Red 5 **Brucella Case Number:** B16-0072 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 6 **Animal ID:** Red 6 **Brucella Case Number:** B16-0073 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 7 **Animal ID:** Red 7 **Brucella Case Number:** B16-0074 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 8 **Animal ID:** Red 8 **Brucella Case Number:** B16-0075 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 9 **Animal ID:** Red 9 **Brucella Case Number:** B16-0076 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 11 **Animal ID:** Red 11 **Brucella Case Number:** B16-0077 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 13 **Animal ID:** Red 13 **Brucella Case Number:** B16-0078 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 14 **Animal ID:** Red 14 **Brucella Case Number:** B16-0079 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 16 **Animal ID:** Red 16 **Brucella Case Number:** B16-0080 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 17 **Animal ID:** Red 17 **Brucella Case Number:** B16-0081 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 18 **Animal ID:** Red 18 **Brucella Case Number:** B16-0082 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 19 **Animal ID:** Red 19 **Brucella Case Number:** B16-0083 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 20 **Animal ID:** Red 20 **Brucella Case Number:** B16-0084 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 21 **Animal ID:** Red 21 **Brucella Case Number:** B16-0085 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 22 **Animal ID:** Red 22 **Brucella Case Number:** B16-0086 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 24 **Animal ID:** Red 24 **Brucella Case Number:** B16-0087 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 25 **Animal ID:** Red 25 **Brucella Case Number:** B16-0088 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 26 **Animal ID:** Red 26 **Brucella Case Number:** B16-0089 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 27 **Animal ID:** Red 27 **Brucella Case Number:** B16-0090 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 28 **Animal ID:** Red 28 **Brucella Case Number:** B16-0091 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 29 **Animal ID:** Red 29 **Brucella Case Number:** B16-0092 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 31 **Animal ID:** Red 31 **Brucella Case Number:** B16-0093 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

[Help Us Help You](#)

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Tuesday, March 22, 2016 2:38:01 PM
Attachments: [16-004824_DBL-BRUC_ALT_03-22-2016-01-10-39-PM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, March 22, 2016 2:10 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: FW: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Tuesday, March 22, 2016 12:12 PM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-004824,Purpose:GEN_DIAG,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-004824

Date Received: 02/10/2016 11:06:25 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner *W i l d l i f e*

USDA, APHIS, VS- GonaCon Study
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number: **16-004824**

Date Collected:

Date Received: 02/10/2016

Date Completed:

Collected By: Clarke, Frey

Purpose: General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Green 2 Animal ID: Green 2 Brucella Case Number: B16-0059 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 3 Animal ID: Green 3 Brucella Case Number: B16-0060 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 4 Animal ID: Green 4 Brucella Case Number: B16-0061 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 6 **Animal ID:** Green 6 **Brucella Case Number:** B16-0062 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Green 8 **Animal ID:** Green 8 **Brucella Case Number:** B16-0063 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 9 **Animal ID:** Green 9 **Brucella Case Number:** B16-0064 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 14 **Animal ID:** Green 14 **Brucella Case Number:** B16-0065 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 15 **Animal ID:** Green 15 **Brucella Case Number:** B16-0066 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Green 17 **Animal ID:** Green 17 **Brucella Case Number:** B16-0067 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 1 **Animal ID:** Red 1 **Brucella Case Number:** B16-0068 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 2 **Animal ID:** Red 2 **Brucella Case Number:** B16-0069 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 3 **Animal ID:** Red 3 **Brucella Case Number:** B16-0070 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 4 **Animal ID:** Red 4 **Brucella Case Number:** B16-0071 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 5 **Animal ID:** Red 5 **Brucella Case Number:** B16-0072 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 6 **Animal ID:** Red 6 **Brucella Case Number:** B16-0073 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 7 **Animal ID:** Red 7 **Brucella Case Number:** B16-0074 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 8 **Animal ID:** Red 8 **Brucella Case Number:** B16-0075 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 9 **Animal ID:** Red 9 **Brucella Case Number:** B16-0076 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 11 **Animal ID:** Red 11 **Brucella Case Number:** B16-0077 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 13 **Animal ID:** Red 13 **Brucella Case Number:** B16-0078 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 14 **Animal ID:** Red 14 **Brucella Case Number:** B16-0079 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 16 **Animal ID:** Red 16 **Brucella Case Number:** B16-0080 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 17 **Animal ID:** Red 17 **Brucella Case Number:** B16-0081 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 18 **Animal ID:** Red 18 **Brucella Case Number:** B16-0082 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 19 **Animal ID:** Red 19 **Brucella Case Number:** B16-0083 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 20 **Animal ID:** Red 20 **Brucella Case Number:** B16-0084 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 21 **Animal ID:** Red 21 **Brucella Case Number:** B16-0085 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 22 **Animal ID:** Red 22 **Brucella Case Number:** B16-0086 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 24 **Animal ID:** Red 24 **Brucella Case Number:** B16-0087 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 25 **Animal ID:** Red 25 **Brucella Case Number:** B16-0088 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 26 **Animal ID:** Red 26 **Brucella Case Number:** B16-0089 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 27 **Animal ID:** Red 27 **Brucella Case Number:** B16-0090 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 28 **Animal ID:** Red 28 **Brucella Case Number:** B16-0091 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 29 **Animal ID:** Red 29 **Brucella Case Number:** B16-0092 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red 31 **Animal ID:** Red 31 **Brucella Case Number:** B16-0093 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

[Help Us Help You](#)

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Thursday, March 31, 2016 9:54:28 AM
Attachments: [16-009019_DBL-BRUC_ALT_03-31-2016-09-28-25-AM.pdf](#)

FYI

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 31, 2016 8:46 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, March 31, 2016 8:33 AM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-009019

Date Received: 03/17/2016 10:31:21 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA APHIS VS- GonaCon
Corwin Springs, MT

Accession Number: 16-009019

Animal Location

Park County MT

Date Collected:

Date Received: 03/17/2016

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 03/31/2016

Collected By: Clarke, Fray

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red43 **Animal ID:** Red43 **Brucella Case Number:** B16-0117 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Not Identified

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Suspect

Colony estimates: Milk Swab: 10-50, Vaginal Swab: >300, Vaginal Discharge: Confluent growth, Feces: 10-50.

Sample: Red13 **Animal ID:** Red13 **Brucella Case Number:** B16-0118 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Feces / Feces

Brucella Isolation Result	Contaminated
---------------------------	--------------

Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300.**Sample:** Green14 **Animal ID:** Green14 **Brucella Case Number:** B16-0119 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Milk / Milk

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Feces / Feces

Brucella Isolation Result	Suspect
---------------------------	---------

Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300, Milk: 1 colony, Feces: 1 colony, but contaminated.

Sample: 3G14 **Animal ID:** 3G14 **Brucella Case Number:** B16-0120 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Milk / Milk

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Suspect

Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300, Milk: 10-50, Feces: 1 colony, but contaminated.**Results authorized by:** Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**[Help Us Help You](#)**

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov
Date: Thursday, March 31, 2016 9:54:28 AM
Attachments: [16-009019_DBL-BRUC_ALT_03-31-2016-09-28-25-AM.pdf](#)

FYI

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 31, 2016 8:46 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist -GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, March 31, 2016 8:33 AM
To: Clarke, Patrick R. - APHIS
Subject: NVSL Report - Accession#16-009019,Purpose:DEV_RES,Exam Req:BRUC sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 16-009019

Date Received: 03/17/2016 10:31:21 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA APHIS VS- GonaCon
Corwin Springs, MT

Accession Number: 16-009019

Animal Location

Park County MT

Date Collected:

Date Received: 03/17/2016

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 03/31/2016

Collected By: Clarke, Fray

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red43 **Animal ID:** Red43 **Brucella Case Number:** B16-0117 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Not Identified

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Suspect

Colony estimates: Milk Swab: 10-50, Vaginal Swab: >300, Vaginal Discharge: Confluent growth, Feces: 10-50.

Sample: Red13 **Animal ID:** Red13 **Brucella Case Number:** B16-0118 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Feces / Feces

Brucella Isolation Result	Contaminated
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Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300.**Sample:** Green14 **Animal ID:** Green14 **Brucella Case Number:** B16-0119 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Milk / Milk

Brucella Isolation Result	Isolate Determined
Brucella Identification Result	Brucella abortus

Feces / Feces

Brucella Isolation Result	Suspect
---------------------------	---------

Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300, Milk: 1 colony, Feces: 1 colony, but contaminated.

Sample: 3G14 **Animal ID:** 3G14 **Brucella Case Number:** B16-0120 **Specimen Type:** MILK, TISSUE **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Fluid / Discharge, Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Milk / Milk

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Suspect

Colony Estimates: Vaginal Swab: >300, Vaginal Discharge: >300, Milk: 10-50, Feces: 1 colony, but contaminated.**Results authorized by:** Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**[Help Us Help You](#)**

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Barfield, JENNIFER \(Jennifer.Barfield@ColoState.EDU\)](#); [Wehtje, Morgan E - APHIS](#)
Subject: FW: NVSL Report - Accession#16-022848,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:27:49 PM
Attachments: [16-022848_DBL-BRUC_ALT_10-05-2016-01-02-49-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:03 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: NVSL Report - Accession#16-022848,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 16-022848

Date Received: 07/15/2016 09:35:48 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS
Fort Collins, CO

Animal Location
CO

Submitter - 2649
Dr. Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140

Accession Number: 16-022848

Date Collected: 03/29/2016

Date Received: 07/15/2016

Date Completed: 10/05/2016

Collected By: Barfield

Purpose: NVSL Internal

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: E1 Animal ID: 91 embryos Brucella Case Number: B16-0439 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E2 Animal ID: 78 embryos Brucella Case Number: B16-0441 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E3 Animal ID: 48 embryos Brucella Case Number: B16-0442 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E4 Animal ID: 129 embryos **Brucella Case Number:** B16-0443 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E5 Animal ID: 95 embryos **Brucella Case Number:** B16-0444 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M1 Animal ID: 1ml of combined C2 from E1 **Brucella Case Number:** B16-0445 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M2 Animal ID: 1ml of combined C2 from E2 **Brucella Case Number:** B16-0446 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M3 Animal ID: 1ml of combined C2 from E3 **Brucella Case Number:** B16-0447 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M4 Animal ID: 1ml of combined C2 from E4 **Brucella Case Number:** B16-0448 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M5 **Animal ID:** 1ml of combined C2 from E5 **Brucella Case Number:** B16-0449 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Barfield, JENNIFER \(Jennifer.Barfield@ColoState.EDU\)](#); [Wehtje, Morgan E - APHIS](#)
Subject: FW: NVSL Report - Accession#16-022848,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:27:49 PM
Attachments: [16-022848_DBL-BRUC_ALT_10-05-2016-01-02-49-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:03 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: NVSL Report - Accession#16-022848,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 16-022848

Date Received: 07/15/2016 09:35:48 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]



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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS
Fort Collins, CO

Animal Location
CO

Submitter - 2649
Dr. Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140

Accession Number: 16-022848

Date Collected: 03/29/2016

Date Received: 07/15/2016

Date Completed: 10/05/2016

Collected By: Barfield

Purpose: NVSL Internal

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: E1 Animal ID: 91 embryos Brucella Case Number: B16-0439 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E2 Animal ID: 78 embryos Brucella Case Number: B16-0441 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E3 Animal ID: 48 embryos Brucella Case Number: B16-0442 Specimen Type: Embryo Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E4 Animal ID: 129 embryos **Brucella Case Number:** B16-0443 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: E5 Animal ID: 95 embryos **Brucella Case Number:** B16-0444 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M1 Animal ID: 1ml of combined C2 from E1 **Brucella Case Number:** B16-0445 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M2 Animal ID: 1ml of combined C2 from E2 **Brucella Case Number:** B16-0446 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M3 Animal ID: 1ml of combined C2 from E3 **Brucella Case Number:** B16-0447 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M4 Animal ID: 1ml of combined C2 from E4 **Brucella Case Number:** B16-0448 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: M5 **Animal ID:** 1ml of combined C2 from E5 **Brucella Case Number:** B16-0449 **Specimen Type:** Embryo **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Embryo / Tissue- Not Identified

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Wehtje, Morgan E - APHIS](#)
Subject: FW: NVSL Report - Accession#16-022849,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:28:55 PM
Attachments: [16-022849_DBL-BRUC_ALT_10-05-2016-01-02-50-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:03 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: NVSL Report - Accession#16-022849,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 16-022849

Date Received: 07/15/2016 09:44:02 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]



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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS

Fort Collins, CO

Animal Location

CO

Submitter - 2649

Dr. Jack C. Rhyan

USDA, APHIS, VS

National Wildlife Research Center

4101 La Porte Ave

Fort Collins, CO 80521

FAX #: 970-266-6138

Phone #: 970-266-6140

Accession Number:

16-022849

Date Collected:

07/11/2016

Date Received:

07/15/2016

Date Completed:

10/05/2016

Collected By:

McCollum

Purpose:

NVSL Internal

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Animal ID: 26 Brucella Case Number: B16-0440 Specimen Type: UTERS, MILK Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

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From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Wehtje, Morgan E - APHIS](#)
Subject: FW: NVSL Report - Accession#16-022849,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:28:55 PM
Attachments: [16-022849_DBL-BRUC_ALT_10-05-2016-01-02-50-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:03 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: NVSL Report - Accession#16-022849,Purpose:NVSL_INTER,Exam Req:BRUC sent to jack.c.rhyan@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 16-022849

Date Received: 07/15/2016 09:44:02 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: CO

Species: [Bison]



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Ames, Iowa 50010

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS

Fort Collins, CO

Animal Location

CO

Submitter - 2649

Dr. Jack C. Rhyan

USDA, APHIS, VS

National Wildlife Research Center

4101 La Porte Ave

Fort Collins, CO 80521

FAX #: 970-266-6138

Phone #: 970-266-6140

Accession Number:

16-022849

Date Collected:

07/11/2016

Date Received:

07/15/2016

Date Completed:

10/05/2016

Collected By:

McCollum

Purpose:

NVSL Internal

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Animal ID: 26 Brucella Case Number: B16-0440 Specimen Type: UTERS, MILK Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 1:08:01 PM
Attachments: [16-025296_DBL-BRUC_ALT_10-05-2016-01-32-40-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:46 PM
To: Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline nol@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number: First Cohort

FAD Number:

Accession: 16-025296

Date Received: 08/05/2016 11:09:32 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BABORT

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



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Ames, Iowa 50010

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA APHIS VS- GonaCon Study
Corwin Springs, MT

Accession Number: 16-025296

Animal Location

Park County MT

Date Collected:

Date Received: 08/05/2016

Submitter - 1961

Dr. Patrick Ryan Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Date Completed: 10/05/2016

Collected By: Frey, Clarke

Purpose: NVSL Internal

Referral Number: First Cohort

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 5G09 **Animal ID:** 5G09 **Brucella Case Number:** B16-0489 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 5R02 **Animal ID:** 5R02 **Brucella Case Number:** B16-0490 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B16-0491 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red28 Animal ID: Red28 Brucella Case Number: B16-0492 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red1 Animal ID: Red1 Brucella Case Number: B16-0493 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red5 Animal ID: Red5 Brucella Case Number: B16-0494 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red11 Animal ID: Red11 Brucella Case Number: B16-0495 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red14 Animal ID: Red14 Brucella Case Number: B16-0496 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red4 Animal ID: Red4 Brucella Case Number: B16-0497 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B16-0498 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B16-0499 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red25 **Animal ID:** Red25 **Brucella Case Number:** B16-0500 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B16-0501 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 1:08:01 PM
Attachments: [16-025296_DBL-BRUC_ALT_10-05-2016-01-32-40-PM.pdf](#)

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, October 05, 2016 12:46 PM
To: Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline nol@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number: First Cohort

FAD Number:

Accession: 16-025296

Date Received: 08/05/2016 11:09:32 AM

Purpose: NVSL Interlab Diagnostic

Exam(s) Requested: BABORT

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA APHIS VS- GonaCon Study
Corwin Springs, MT

Accession Number: 16-025296

Animal Location

Park County MT

Date Collected:

Date Received: 08/05/2016

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 10/05/2016

Collected By: Frey, Clarke

Purpose: NVSL Internal

Referral Number: First Cohort

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 5G09 **Animal ID:** 5G09 **Brucella Case Number:** B16-0489 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 5R02 **Animal ID:** 5R02 **Brucella Case Number:** B16-0490 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B16-0491 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red28 Animal ID: Red28 Brucella Case Number: B16-0492 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red1 Animal ID: Red1 Brucella Case Number: B16-0493 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red5 Animal ID: Red5 Brucella Case Number: B16-0494 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red11 Animal ID: Red11 Brucella Case Number: B16-0495 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red14 Animal ID: Red14 Brucella Case Number: B16-0496 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red4 Animal ID: Red4 Brucella Case Number: B16-0497 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B16-0498 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B16-0499 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red25 **Animal ID:** Red25 **Brucella Case Number:** B16-0500 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B16-0501 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov
Date: Friday, September 23, 2016 11:24:01 AM
Attachments: [16-025298_DBL-BRUC_ALT_09-22-2016-09-04-59-AM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, September 22, 2016 9:03 AM
To: Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: FW: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov

R20, G08

P. Ryan Clarke, DVM, MPH
USDA, APHIS, VS, SPRS
District 5 Epidemiologist-GYA
Bozeman, Montana
406-388-5162

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Thursday, September 22, 2016 8:05 AM
To: Clarke, Patrick R. - APHIS <Patrick.R.Clarke@aphis.usda.gov>
Subject: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number: Cohort #1

FAD Number:

Accession: 16-025298

Date Received: 08/05/2016 11:16:21 AM

Purpose: General Diagnostic

Exam(s) Requested: BABORT

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon
Corwin Springs, MT**Accession Number:** 16-025298**Animal Location**

Park County MT

Date Collected: 07/21/2016**Date Received:** 08/05/2016**Submitter - 1961**Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162**Date Completed:** 09/21/2016**Collected By:** R. Frey, P.R. Clarke**Purpose:** General Diagnostic**Referral Number:** Cohort #1**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Red20 **Animal ID:** Red20 **Brucella Case Number:** B16-0527 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Green08 **Animal ID:** Green08 **Brucella Case Number:** B16-0528 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by:Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov
Date: Friday, September 23, 2016 11:24:01 AM
Attachments: [16-025298_DBL-BRUC_ALT_09-22-2016-09-04-59-AM.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, September 22, 2016 9:03 AM
To: Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
Subject: FW: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov

R20, G08

P. Ryan Clarke, DVM, MPH
USDA, APHIS, VS, SPRS
District 5 Epidemiologist-GYA
Bozeman, Montana
406-388-5162

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Sent: Thursday, September 22, 2016 8:05 AM
To: Clarke, Patrick R. - APHIS <Patrick.R.Clarke@aphis.usda.gov>
Subject: NVSL Report - Accession#16-025298,Purpose:GEN_DIAG,Exam Req:BABORT sent to patrick.r.clarke@aphis.usda.gov

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number: Cohort #1

FAD Number:

Accession: 16-025298

Date Received: 08/05/2016 11:16:21 AM

Purpose: General Diagnostic

Exam(s) Requested: BABORT

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon
Corwin Springs, MT**Accession Number:** 16-025298**Animal Location**

Park County MT

Date Collected: 07/21/2016**Date Received:** 08/05/2016**Submitter - 1961**Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162**Date Completed:** 09/21/2016**Collected By:** R. Frey, P.R. Clarke**Purpose:** General Diagnostic**Referral Number:** Cohort #1**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Red20 **Animal ID:** Red20 **Brucella Case Number:** B16-0527 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Green08 **Animal ID:** Green08 **Brucella Case Number:** B16-0528 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by:Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: FW: permission to bring bison from MT quarantine facility
Date: Wednesday, January 07, 2015 5:09:08 PM

FYI

From: Roehr - CDA, Keith [mailto:keith.roehr@state.co.us]
Sent: Wednesday, January 07, 2015 3:49 PM
To: Rhyan, Jack C - APHIS
Subject: Re: permission to bring bison from MT quarantine facility
Jack,

Yes please have the DVM in MT complete a CVI and the VS 1-27 as well.

Keith A. Roehr DVM
State Veterinarian



P 303.869.9130 | F 303.466.8515 |
305 Interlocken Parkway, Broomfield, CO 80021
keith.roehr@state.co.us | www.colorado.gov/ag
On Wed, Jan 7, 2015 at 3:43 PM, Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
wrote:

Keith,

May we bring approximately 14 bison (see below) from Montana into our facility next
Thursday or possibly Friday (Jan 15 or 16)? We will do it on a 127.

Jack

From: Frey, Rebecca K - APHIS
Sent: Wednesday, December 24, 2014 10:54 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Cc: Clarke, Patrick R. - APHIS
Subject: confirmation of animals to CO

By my count as of today.....and we have received almost all of the results from NVSL to
help with the decision making.....You all will be taking 7 2014 calves, 4 seronegative cows
and an unknown number....but presumably about 3..... cows that were injected with
GonaCon in May 2014 but are pregnant.
Bring your big trailer..... ☺

This electronic message contains information generated by the USDA solely for the intended recipients. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the violator to civil or criminal penalties. If you believe you have received this message in error, please notify the sender and delete the email immediately.

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: permit
Date: Monday, February 24, 2014 2:15:18 PM
Attachments: [APHIS_BisonTransferAgreement_Feb2013\(1\).docx](#)
[APHIS_BisonTransferAgreement_Feb2013MM.docx](#)

Here is an initial stab at rewriting the permit. Both the old GnRH one and the new embryo transfer one are attached.

Please take a look and hack it up... Animal numbers?

Matt

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 1:51 PM
To: McCollum, Matthew P - APHIS
Subject: permit
Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

INTERAGENCY AGREEMENT
between the
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
and the
NATIONAL PARK SERVICE

ARTICLE I. BACKGROUND AND OBJECTIVES

To evaluate sterilization by use of GonaCon™, an immunocontraceptive vaccine, and ovariectomy as means of decreasing the potential for transmission of *Brucella abortus* in bison. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

ARTICLE II. STATEMENT OF WORK

A. During the period of performance, up to 63 live bison (8-16 seronegative bulls, 32-40 seropositive cows, 5-7 seronegative cows) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to determine whether:

- Immunocontraception and/or ovariectomy procedures can prevent the shedding of *Brucella abortus* bacteria in young, recently infected bison;
- Immunocontraception with GonaCon™ vaccine can prevent shedding of *Brucella abortus* bacteria throughout the infection cycle;
- Recovery from the contraceptive treatment and the brucellosis infection can be completed without any further shedding of the bacteria during subsequent pregnancies; and
- Behavioral changes occur during the breeding season when females are treated with two types of pregnancy prevention procedures.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

D. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

ARTICLE III. TERM OF AGREEMENT

The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

ARTICLE IV. KEY OFFICIALS

National Park Service
Yellowstone Center for Resources
Rick Wallen, Wildlife Biologist

Animal and Plant Health Inspection Service
Veterinary Services
Jack Rhyan, DVM

P.O. Box 168
Yellowstone National Park, WY 82190
307-344-2285
rick_wallen@nps.gov

National Wildlife Research Center
Fort Collins, CO 80521
970-266-6140
Jack.C.Rhyan@aphis.usda.gov

ARTICLE V. PAYMENT

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status. Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure should be consigned to a quarantine location for further diagnostics, directly to a managed for public trust conservation program to supplement population genetic diversity, to an introduction program to establish a new conservation population of wild bison, or if no such opportunities exist, to a private not-for-profit bison conservation program. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis-free bison to any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

ARTICLE VII. PRIOR APPROVAL

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq., 16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in the same research study at the same location.

ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

ARTICLE IX. TERMINATION

Either party may terminate the agreement by providing 14 days advance written notice to the other party.

ARTICLE X. AUTHORIZING SIGNATURES

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: _____
Name: Daniel N. Wenk
Title: Superintendent, Yellowstone NP
Date: February ____, 2013

Signature: _____
Name: ??????????
Title: ??????????
Date: February ____, 2013

Signature: _____
Name: Tina Holland
Title: Contracting Officer
Date: February ____, 2013

Signature: _____
Name: ??????????
Title: ??????????
Date: February ____, 2013

INTERAGENCY AGREEMENT
between the
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
and the
NATIONAL PARK SERVICE

ARTICLE I. BACKGROUND AND OBJECTIVES

To evaluate the use of assisted reproduction techniques as means of genetic preservation for bison that are infected with *Brucella abortus*. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

ARTICLE II. STATEMENT OF WORK

A. During the period of performance, up to 15 live bison (12 adult cows and 3 adult bulls) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to evaluate embryos, offspring, and recipients for transmission of brucellosis via embryo transfer when in vivo and in vitro produced embryos are generated from cows and bulls with various titers of *Brucella abortus*. The rationale for this experiment is proof of principle that brucellosis-free embryos can be generated using oocytes and semen from infected animals without transmission of disease to embryo recipients or offspring.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

D. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

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The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

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National Park Service
Yellowstone Center for Resources
Rick Wallen, Wildlife Biologist
P.O. Box 168
Yellowstone National Park, WY 82190
307-344-2285
rick_wallen@nps.gov

Animal and Plant Health Inspection Service
Veterinary Services
Jack Rhyan, DVM
National Wildlife Research Center
Fort Collins, CO 80521
970-266-6140
Jack.C.Rhyan@aphis.usda.gov

ARTICLE V. PAYMENT

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status. Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure should be consigned to a quarantine location for further diagnostics, directly to a managed for public trust conservation program to supplement population genetic diversity, to an introduction program to establish a new conservation population of wild bison, or if no such opportunities exist, to a private not-for-profit bison conservation program. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis-free bison to any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

ARTICLE VII. PRIOR APPROVAL

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq., 16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in the same research study at the same location.

ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

ARTICLE IX. TERMINATION

Either party may terminate the agreement by providing 14 days advance written notice to the other party.

ARTICLE X. AUTHORIZING SIGNATURES

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: _____
Name: Daniel N. Wenk
Title: Superintendent, Yellowstone NP
Date: February ____, 2013

Signature: _____
Name: ??????????
Title: ??????????
Date: February ____, 2013

Signature: _____
Name: Tina Holland
Title: Contracting Officer
Date: February ____, 2013

Signature: _____
Name: ??????????
Title: ??????????
Date: February ____, 2013

From: [Matt McCollum](#)
To: Pauline.Nol@aphis.usda.gov
Cc: [Jason E Lombard](#)
Subject: Fw: plot gets thicker.....
Date: Wednesday, March 30, 2011 11:17:00 AM

Hey Pauline,

Here is more from Jason.

THANKS JASON!!!

Matt

----- Forwarded by Matt McCollum/CO/APHIS/USDA on 03/30/2011 11:16 AM -----

**Jason E
Lombard/CO/APHIS/USDA**

03/30/2011 10:04 AM

ToMatt

McCollum/CO/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA,
Rebecca K
Frey/MT/APHIS/USDA@USDA

cc

SubjectRe: Fw: plot gets thicker.....



Hi Matt, Jack and Becky,

I didn't see Pauline's email address in the address book, so please forward this to her as well.


As I mentioned to Jack this morning, these sample sizes are for the seronegative group. You can modify the seropositive groups size to what you think is appropriate to cause seroconversion in Pasture B, assuming Pasture A will have little or no seroconversion anyway.

Pasture A Seroconversion	Sample size per group				
0.5	407	103	45	24	14
0.4	107	49	28	17	11
0.3	49	29	19	13	9
0.2	28	19	13	10	8
0.1	17	13	10	8	6
0.01	11	9	8	6	5
	0.6	0.7	0.8	0.9	0.99
	Pasture B Seroconversion				

Between 15 and 20 in each seronegative group seems like plenty based on what you have told me. I look forward to continue working on this project.

Cheers!
Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

 Matt McCollum/CO/APHIS/USDA

**Matt
McCollum/CO/APHIS/USDA**

03/18/2011 10:53 AM

ToRebecca.K.Frey@aphis.usda.gov

ccJason E

Lombard/CO/APHIS/USDA@USDA

SubjectRe: Fw: plot gets thicker.....




Hey Becky,

I talked with Jason and he says he can take a look at it. Hopefully he can get something together by mid week next.

THANKS JASON!!!

Matt

 Rebecca K Frey/MT/APHIS/USDA

**Rebecca K
Frey/MT/APHIS/USDA**

03/18/2011 09:33 AM

ToMatt McCollum/CO/APHIS/USDA@USDA

cc

SubjectFw: plot gets thicker.....

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell

----- Forwarded by Rebecca K Frey/MT/APHIS/USDA on 03/18/2011 09:32 AM -----

**Pauline
Nol/CO/APHIS/USDA**

03/09/2011 02:47 PM

ToRebecca K Frey/MT/APHIS/USDA@USDA

cc

Subjectplot gets thicker.....

Hey Becky,

I talked to Jenny Powers and she sent me the latest comments from her and Margaret Wild. This is getting way complicated actually so I don't see a realistic finalization of this for a while.

Both Margaret and Jenny agreed that having the third sterilized group would be really good. In fact, a better alternative to spayed animals would be tubally ligated animals. Then they would at least have all of their parts and be comparable to the other groups. The only difference is that they will be more likely to actually have sex, as opposed to the GnRH animals.

They were very concerned about the numbers being used, and I have to agree, since the magic number 10 isn't all that magical and we need to have more substance behind our choice of sample size. I am working on that but probably need help on that. The one main thing is that I don't know what our space and financial limits are. Would you be able to give a fixed limit on numbers of animals we can have in the pastures and what we can afford to feed? I don't even know how we are paying for this study.

There are many comments on wanting to carry the animals out further than what the protocol now suggests, which would be great to evaluate the vaccine, but don't know how financially or logistically feasible this would be. Maybe NPS could chip in if they really want to get those data points???

This has the potential of being a very good, but much more complicated study than it started out as. I feel that if we are going to put the effort into it, we should learn as much as we can, and answer NPS's questions, since they are the people we are trying to convince to use the vaccine, if it looks feasible. But, this can become a huge monster too.

I have to go pick up kiddies now but I will work some more on this tomorrow.
Pauline

[attachment "Rhyon Immunocontraception Study Plan_rlw review brmd.doc" deleted by Matt McCollum/CO/APHIS/USDA]

From: [Jack C Rhyan](#)
To: [Pauline Nol](#)
Subject: Fw: plot gets thicker.....
Date: Wednesday, March 30, 2011 11:22:00 AM

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 03/30/2011 11:22 AM -----

**Jason E
Lombard/CO/APHIS/USDA**

03/30/2011 10:04 AM

ToMatt

McCollum/CO/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA,
Rebecca K
Frey/MT/APHIS/USDA@USDA

cc

SubjectRe: Fw: plot gets thicker.....



Hi Matt, Jack and Becky,

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Between 15 and 20 in each seronegative group seems like plenty based on what you have told me. I look forward to continue working on this project.

Cheers!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

 Matt McCollum/CO/APHIS/USDA

**Matt
McCollum/CO/APHIS/USDA**

03/18/2011 10:53 AM

ToRebecca.K.Frey@aphis.usda.gov

ccJason E

Lombard/CO/APHIS/USDA@USDA

SubjectRe: Fw: plot gets thicker.....




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THANKS JASON!!!

Matt

 Rebecca K Frey/MT/APHIS/USDA

**Rebecca K
Frey/MT/APHIS/USDA**

03/18/2011 09:33 AM

ToMatt McCollum/CO/APHIS/USDA@USDA

cc

SubjectFw: plot gets thicker.....

Rebecca Frey, Wildlife Biologist/Disease Specialist

USDA APHIS VS

Bozeman, Montana

(406) 333-4425

(b) (6) cell

----- Forwarded by Rebecca K Frey/MT/APHIS/USDA on 03/18/2011 09:32 AM -----

**Pauline
Nol/CO/APHIS/USDA**

03/09/2011 02:47 PM

ToRebecca K Frey/MT/APHIS/USDA@USDA

cc

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[attachment "Rhyan Immunocontraception Study Plan_rlw review brmd.doc" deleted by Matt McCollum/CO/APHIS/USDA]

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: pregnancy info
Date: Wednesday, June 17, 2015 4:29:18 PM
Attachments: [Pregnancy_GC2015.xlsx](#)

interesting

From: Frey, Rebecca K - APHIS
Sent: Wednesday, June 17, 2015 11:36 AM
To: Rhyan, Jack C - APHIS
Subject: pregnancy info

Jack!

Turns out...I have been keeping up with my data crunching....just so busy I can't remember doing it!!!!!!

So, you can see where each group breaks on this chart. First one is the control pen. 18/20 pregnant.

7 of 16 in GC treatment 1; 2/11 GC treated animals.

GC treatment 2, 5/26; 1 of 20 GC treated animals.

Any missing results are because we don't have them yet.

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

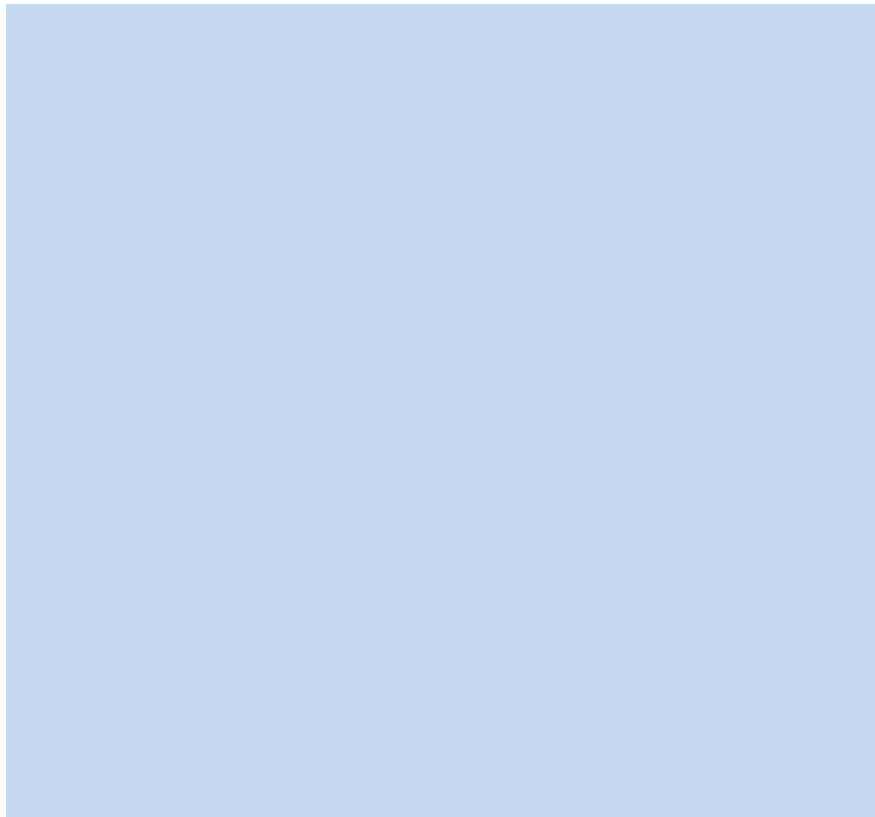
USDA APHIS VS

Montana

406-333-4425 office/fax

Bangle Tag	GonaCon	Pregnant 2013	Birth	Serology	Culture
Green 08	No	Yes	Calf	Neg	Neg
Green 09	No	Yes	Weak Calf	Pos	Pos
Green 10	No	Yes	Weak Calf/Abortion	Pos	Pos
Green 14	No	Yes	Calf	Neg	Neg
Green 15	No	Yes	Abortion (mummy)	Pos	Neg
Red 03	No	Yes	Abortion	Pos	Pos
Red 06	No	Yes	None, removed radio	Pos	Neg
Red 07	No	Yes	Calf	Pos	Neg
Red 08	No	Yes	Calf	Pos	Neg
Red 09	No	No	n/a	Pos	Neg
Red 13	No	Yes	Calf	Pos	Pos
Red 15	No	No	n/a	Pos	Neg
Red 16	No	Yes	Abortion	Pos	Pos
Red 17	No	No	n/a	Pos	Neg
Red 18	No	Yes	Calf	Pos	Neg
Red 20	Yes	Yes	Calf	Pos	Neg
Red 21	No	Yes	Calf	Pos	Pos
Red 22	No	Yes	Calf	Pos	Neg
Red 25	No	Yes	Calf	Pos	Neg
Red 24	Yes	Yes	Calf	Pos	Neg
Red 26	Yes	Yes	Calf	Pos	Neg
Red 30	No	Yes	Calf	Pos	Neg
GONACON PEN 1					
Green 02	No	Yes	Calf	Neg	Neg
Green 03	No	Yes	Calf	Neg	Neg
Green 04	No	No	n/a	Neg	Neg
Green 06	No	No	n/a	Sus	Neg
Green 17	No	Yes	Calf	Neg	Neg
Red 01	Yes	No	n/a	Neg	Neg
Red 02	Yes	No	n/a	Pos	Neg
Red 04	Yes	No	n/a	Neg	Neg
Red 05	Yes	No	n/a	Pos	Neg
Red 11	Yes	No	n/a	Neg	Neg
Red 14	Yes	No	n/a	Pos	Neg
Red 19	Yes	No	n/a	Pos	Neg
Red 23	Yes	No	n/a	Pos	Neg
Red 27	Yes	No	n/a	Neg	Neg
Red 28	Yes	No	n/a	Pos	Neg
Red 29	Yes	No	n/a	Pos	Neg
Red 31	Yes	No	n/a	Neg	Neg
GONACON PEN 2					
Green 01	No				
Green 07	No				
Green 13	No				
Green 21	No				
Green 24	No				

Green 25	No
Red 32	Yes
Red 34	Yes
Red 36	Yes
Red 38	Yes
Red 39	Yes
Red 41	Yes
Red 42	Yes
Red 43	Yes
Red 44	Yes
Red 45	Yes
Red 46	Yes
Red 47	Yes
Red 48	Yes
Red 49	Yes
Red 51	Yes
Red 52	Yes
Red 53	Yes
Red 54	Yes
Red 55	Yes
Red 56	Yes



Pregnant 2014	Birth	Serology	Culture	Pregnant 2015	Birth	Serology
Yes	Calf	Neg	neg	Yes	Calf	Neg
No	n/a	Pos	neg	Yes	Calf	Pos
Yes	Abortion	Pos	Neg/strep.	Yes	Mummy/Cow died 5/18,	
Yes	Abortion	Pos	Pos	Yes	Abortion	Pos
Yes	Abortion	Pos	Pos	Yes	Calf	Pos
Yes	Calf	Pos	Pos	Yes	Calf	
Yes	Calf	Pos	neg	Yes	Abortion	Pos
Yes	Calf	Sus	neg	Yes	Calf	Neg
Yes	Abortion	Pos	Pos	Yes	Abortion	Pos
No	n/a	Pos	neg	Yes	Stillbirth	Pos
Yes	Calf	Pos	Neg	Yes	Calf	Pos
Yes/Dead	DEAD		DEAD	No samples		
Yes	Calf	Pos	neg	Yes	Calf	Pos
No	n/a	Pos	neg	No	n/a	
No	n/a	Pos	neg	Yes	Calf	Pos
No	n/a	Pos	neg	Yes	Abortion	Pos
Yes	Calf	Pos	neg	Yes	Abortion	Pos
Yes	Calf	Pos	neg	Yes	Calf	
Yes	abortion.mummy	Pos	neg	No	n/a	
Yes	Calf	Pos	Neg	Yes	Calf	Pos
No		Pos	neg	Yes	Calf	Pos
DEAD	DEAD		DEAD	No isolation on Retro or Mammary gland		
Yes	Calf	Neg	neg	Yes	Abortion	
No	n/a	Neg	neg	Yes	Calf	Neg
Yes	Calf/Dead	Neg	neg	Yes	Calf	Neg
Yes	Calf	Neg	neg	Yes	Calf	Neg
Yes	Calf	Neg	neg	Yes	Calf	Neg
No	n/a	Pos	neg	Yes	Calf	Neg
Yes	Calf	Neg	neg			
No	n/a	Pos	neg			
No	n/a	Neg	neg			
No	n/a	Neg	neg			
No	n/a	Pos	neg	Yes	Calf	Pos
No	n/a	Pos	neg			
No/Dead	DEAD		DEAD	B. abortus BV1; retro LN at death		
No	n/a	Pos	neg			
No	n/a	Pos	neg			
No	n/a	Pos	neg			
No	n/a	Neg	neg			
				Yes	Calf	Neg
				Yes	Stillbirth/Hu	Neg
				Yes	Calf	Neg
				No		
				No		

	Yes	Calf
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	
	No	

Culture

/15

Pos
Pos

Neg
Pos

d at death



From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#)
Subject: FW: QA number
Date: Friday, December 30, 2011 10:24:00 AM

Never mind, I found the QA number for this. 1858

Thanks!

From: Nol, Pauline - APHIS
Sent: Friday, December 30, 2011 10:11 AM
To: Greiner, Laura B - APHIS
Subject: QA number



Hi Laura,

I either need a new QA# for the study: **Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

or I need to be reminded of it☺

Thanks and happy New Year!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6138

From: Jenny_Powers@nps.gov
To: pauline.nol
Subject: Fw: Research proposal
Date: Wednesday, April 06, 2011 11:33:00 AM

Hey Pauline,

Just got this from Rick and am about to read. Who is Luke Wagner? Did you really have to put Lowell/Kathy on.....

J

----- Forwarded by Jenny Powers/FTCOLLINS/NPS on 04/06/2011 11:32 AM -----

Rick
Wallen/YELL/NPS

To
04/06/2011 07:51 AM Jenny Powers/FTCOLLINS/NPS@NPS,
Margaret Wild/FTCOLLINS/NPS@NPS

cc
PJ White/YELL/NPS@NPS

Subject
Fw: Research proposal

Jenny, I understand Pauline contacted you to discuss some of our comments regarding the APHIS immunocontraception project. I have not read the revised proposal but hope that your concerns have now been addressed.

I was unable to reach Jack recently because he was out of town but will read through this revised proposal today and contact him if there is further need. I would like to call you later today to check in as well regarding their revisions and whether any further requests are necessary.

We are nearing the end of the time period for handling of live animals in our facility and want to support this research project if we can. APHIS has been helpful this winter agreeing to support some consignment to slaughter for seropositive bison that could be sampled and data used to evaluate our test procedures regarding identification of infectious individuals compared to individuals recovered from infection. They also provided additional fenced winter range that assisted in managing boundary area distribution this winter. Conversations with my colleagues here in Montana have been good in that she (Becky Frey) sees the need to lay out a more rigorous study design and sample long enough to see whether contraceptive vaccinates recover from both brucellosis infection and the

infertility vaccine and subsequently become pregnant and produce offspring and in doing so, fail to shed Brucella bacteria at parturition. The logistical challenges of keeping a research facility under contract are ongoing but look favorable at this time.

Cheers, hope your day is bright. RW

----- Forwarded by Rick Wallen/YELL/NPS on 04/06/2011 07:38 AM -----

Rebecca.K.Frey@aphis.usda.gov

To
04/05/2011 03:58 PM rick_wallen@nps.gov
cc

Subject
Research proposal

Hi Rick,

Here is the edited version 2. We went ahead and added you and Jenny and Margaret as co-investigators, if you don't want to be there, we will let you make the choice.

I had a quick read through, and think that it covers a lot of the initial concerns/comments. If you would prefer, I could initiate the permit, or if you think you could do it quickly I will let you.....Just let me know and I will get on it tomorrow if you think that is best.

Thanks much,
Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell[attachment "ImmunocontBisonProject_4-5-11.doc" deleted by Jenny Powers/FTCOLLINS/NPS]

From: [Jack C Rhyan](#)
To: [Pauline Nol](#); [Matt McCollum](#)
Subject: FW: Sample size
Date: Tuesday, May 10, 2011 4:55:00 PM

FYI

From: Brant Schumaker [mailto:(b) (6)@gmail.com]
Sent: Tuesday, May 10, 2011 4:52 PM
To: Rhyan, Jack C (APHIS)
Subject: Re: Sample size

With 32 animals per group (16 in each pasture) and 30% compared to 10% you're looking at around 64% power. If the treatment group drops to 5% you'll bump up your power to 85%. It shouldn't be a problem to start with two pastures and then bump up to four.

On 5/10/2011 4:44 PM, Rhyan, Jack C (APHIS) wrote:
Brant,

That is very helpful. I anticipate at least 30% abortions or shedders in the non-contracepted bison (young seropositive first calf heifers) and 0-10% in the contracepted bison. (Conservative guesses.) So right now we may write it up with 16 seropositives per pasture and 4 sentinels. I hate to give up the sentinels for the proof of concept.
What do you think. Also, it may be that we start with only 2 pastures (1 control and one treatment) and add 2 more pastures the next year. That will depend on the availability of positive young female bison. Will that be a statistical problem?

Jack

From: Brant Schumaker [mailto:(b) (6)@gmail.com]
Sent: Tuesday, May 10, 2011 3:39 PM
To: Rhyan, Jack C (APHIS)
Subject: Sample size

Hi Jack,

Sample size calculations are somewhat difficult, in that you need to estimate the magnitude of change that you want to be statistically significant and also estimate how close to 50% your start values are.

Some questions that are important:

- What proportion of abortion (or shedding) do you expect in your control group?
- How about in your contracepted group?
- What magnitude of decrease would you like to be statistically significant?

Some sample calculations:

If your max pasture is 80 animals and you divide it equally 40-40 (contracepted/ controls)

You only have 12% power to detect a 5% change (from 50% to 45% abortions)

23% power to detect a 10% change (from 50% to 40% abortions)

If the event is more rare the power goes up

Only 13% power to detect a 5% change (from 30% to 25% abortions)

27% power to detect a 10% change (from 30% to 20% abortions)

With a large effect the power goes up

82% to detect a 22% change (from 30% to 8% abortions)

With sentinels and room for some calves:

Each pen having 14 study animals and 4 controls

Total 28-28 (contracepted/ controls)

Would need to see a 25% change (i.e. 30% to 5% abortions) to have statistical significance.

I think the take-home message is that if you want to show statistical significance it would be best to consider not doing the sentinel portion of the study this go round. That way, even with room for calves (18 animals per pen) you'd be able to have an 82% power for a 23% change (30% to 7% abortions) or 81% for a 28% change (50% to 22%)

Hope this makes sense and feel free to send me the answers to the above questions to make a more informed calculation.

Talk to you soon,

Brant

--

Brant Schumaker DVM, MPVM, PhD
Assistant Professor/ Veterinary Epidemiologist
University of Wyoming
1174 Snowy Range Road
Laramie, WY 82070

(b) (6) [REDACTED]@gmail.com

O: (307) 766-9970

--

Brant Schumaker DVM, MPVM, PhD
Assistant Professor/ Veterinary Epidemiologist
University of Wyoming
1174 Snowy Range Road
Laramie, WY 82070

(b) (6) [REDACTED]@gmail.com

O: (307) 766-9970

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Thursday, July 16, 2015 2:42:12 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 10:36 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Tuesday, June 02, 2015 4:36 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406)994-4885
Fax: (406)994-6344
Email: livdiagnosticlab@mt.gov

Accession # 8-413-15

Client: USDA, APHIS, VS

Submitter: PATRICK RYAN CLARKE D.V.M.
187 TOBIANO TRAIL
BELGRADE MT 59714

Species: WILD - BISON

Breed: BISON

Name/No. G10

Age: ADULT Sex: F

Date Sent:

Date Received 05/18/2015

Preliminary Date 06/02/2015

Preliminary Report

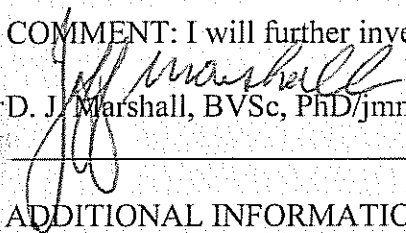
Case Coordinator: JM

CASE SUMMARY

6-02-15 ADDITIONAL INFORMATION:

CLINICAL MICROBIOLOGY: Brucella sp was not isolated from fetal tissue cultures (see attached report).

COMMENT: I will further investigate placenta and the cause of histological changes present.


D. J. Marshall, BVSc, PhD/jmm

ADDITIONAL INFORMATION 6/1/15:

NVSL: Attempts to culture Brucella sp from the tissues submitted from the dam were negative. See attached report.

D. J. Marshall, BVSc, PhD/jmm

REASON FOR SUBMISSION: Bison abortion

LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.

D. J. Marshall, BVSc, PhD/cto

JL Accession #

8-413-15

Submitter:

PATRICK RYAN CLARKE D.V.M.

Owner:

USDA, APHIS, VS

Date In 05/18/2015

PATHOLOGY

Date Out:

Released by: JM

GROSS PATHOLOGY: A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

HISTOPATHOLOGY: Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

MORPHOLOGIC DIAGNOSIS:

Placenta: Placentitis, necrotizing, severe

Date In 05/19/2015

BACTERIOLOGY

Date Out: 06/01/2015 Released by: mh

CULTURES

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	lung	Brucella	Negative for Brucella sp.		NA
	abomasal swab	Brucella	Negative for Brucella sp.		NA
	placenta	Brucella	Negative for Brucella sp.		NA

Date In: 05/19/2015

REFERRAL/OTHER

Date Out: 06/01/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
119	Tissue	Brucella Culture	No isolation made.	NVSL

Please see attached report for complete results.

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Thursday, July 16, 2015 2:40:58 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 10:36 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, June 01, 2015 2:56 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406)994-4885
Fax: (406)994-6344
Email: livdiagnosticlab@mt.gov

Accession #: 8-413-15

Owner: USDA, APHIS, VS

Species: WILD - BISON

Breed: BISON

Date Received: 05/18/2015

Name/No. G10

Preliminary Date 06/01/2015

Age: ADULT Sex: F

Submitter: PATRICK RYAN CLARKE D.V.M.

187 TOBIANO TRAIL

BELGRADE MT 59714

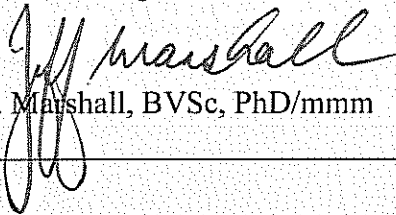
Preliminary Report

Case Coordinator: JM

CASE SUMMARY

ADDITIONAL INFORMATION 6/1/15:

NVSL: Attempts to culture *Brucella* sp from the tissues submitted from the dam were negative.
See attached report.


D. J. Marshall, BVSc, PhD/mmm

REASON FOR SUBMISSION: Bison abortion

LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.

D. J. Marshall, BVSc, PhD\cto

Date In 05/18/2015

PATHOLOGY

Date Out: 05/26/2015

Released by: JM

GROSS PATHOLOGY: A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

HISTOPATHOLOGY: Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

MORPHOLOGIC DIAGNOSIS:

Placenta: Placentitis, necrotizing, severe

002609

MVDL Accession #:
8-413-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA, APHIS, VS

Date In: 05/19/2015

BACTERIOLOGY

Date Out:

Released by:

CULTURES

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	placenta	Brucella	Results Pending		
	lung	Brucella	Results Pending		
	abomasal swab	Brucella	Results Pending		

Date In: 05/19/2015

REFERRAL/OTHER

Date Out: 06/01/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
G10	Tissue	Brucella Culture	No isolation made	NVSL

Please see attached report for complete results.



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA,APHIS,VS
Corwin Springs, MT

Accession Number: 15-016456

Animal Location

Park County MT

Date Collected: 05/18/2015

Date Received: 05/20/2015

Submitter - 2046

MT Department of Livestock
Diagnostic Laboratory Division
1911 W Lincoln St
PO Box 997
Bozeman, MT 59718
FAX #: 406-994-6344
Phone #: 406-994-4885

Date Completed: 06/01/2015

Collected By: Dr. P. Ryan Clarke

Purpose: General Diagnostic

Referral Number: 8-413-15

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 8-413-15 Animal ID: G10 Brucella Case Number: B15-0162 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Tissue / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Tissue / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Lymph Node- Popliteal

Brucella Isolation Result

No Isolation Made

Tissue / Lymph Node- Mandibular

Brucella Isolation Result

No Isolation Made

Tissue / Lymph Node- Parotid

Brucella Isolation Result

No Isolation Made

Tissue / Spleen

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Tissue / Kidney

Brucella Isolation Result

No Isolation Made

Tissue / Lung

Brucella Isolation Result

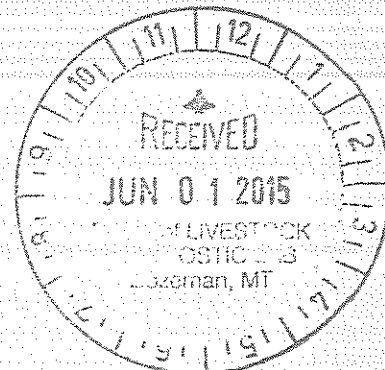
No Isolation Made

Tissue / Mesentery

Brucella Isolation Result

No Isolation Made

Most samples had heavy contamination.



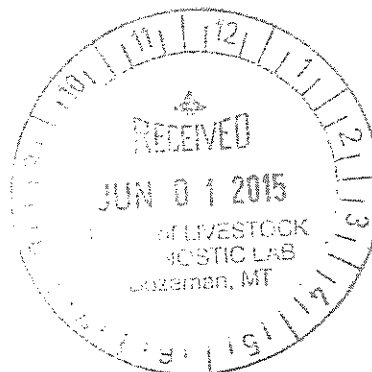
Scanned 6-1-15/jm

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.



From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Thursday, July 16, 2015 2:07:11 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 10:36 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Tuesday, May 26, 2015 5:20 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406) 994-4885
Fax: (406) 994-6344
Email: livdiagnosticlab@mt.gov

Accession # 8-413-15

Owner: USDA, APHIS, VS

Submitter: PATRICK RYAN CLARKE D.V.M.

187 TOBIANO TRAIL

BELGRADE MT 59714

Species: WILD - BISON

Breed: BISON

Name/No. G10

Age: ADULT Sex: F

Date Received 05/18/2015

Preliminary Date 05/26/2015

Preliminary Report

Case Coordinator: JM

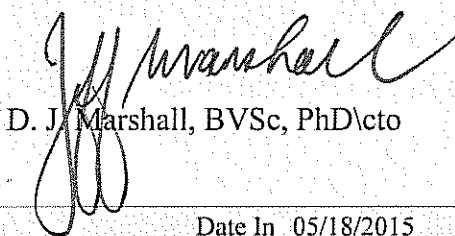
CASE SUMMARY

REASON FOR SUBMISSION: Bison abortion

LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.


D. J. Marshall, BVSc, PhD\cto

Date In 05/18/2015

PATHOLOGY

Date Out: 05/26/2015

Released by: JM

GROSS PATHOLOGY: A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

HISTOPATHOLOGY: Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

MORPHOLOGIC DIAGNOSIS:

Placenta: Placentitis, necrotizing, severe

Date In 05/19/2015

BACTERIOLOGY

Date Out:

Released by:

CULTURES

Antimicrobial
Profile

ID/Site	Specimen	Culture Type	Isolate	Growth
	placenta	Brucella	Results Pending	
	lung	Brucella	Results Pending	
	abomasal swab	Brucella	Results Pending	

MVDL Accession #
8-413-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA, APHIS, VS

Date In: 05/19/2015		REFERRAL/OTHER		Date Out:	Released by: JM
<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>	
	Tissue	Brucella Culture		NVSL	
Results Pending					

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Thursday, July 16, 2015 2:03:37 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 10:31 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

Jack,

Sending you about 5-6 necropsy reports (MT diagnostic lab) in a row.....that I've accumulated over the last month or two (?) and may not have passed on to anyone.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Thursday, May 14, 2015 2:27 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406)994-4885
Fax: (406)994-6344
Email: livdiagnosticlab@mt.gov


Accession #: 8-397-15
Owner: USDA/APHIS/VS

Species: WILD - BISON
Breed: BISON
Name/No. 5G07
Age: NEWB(Sex:

Date Sent: 05/13/2015
Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.
187 TOBIANO TRAIL
BELGRADE MT 59714

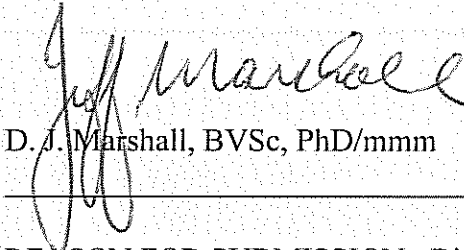
Final Report


Case Coordinator: JM

CASE SUMMARY

CORRECTED REPORT 5/13/15:

BACTERIOLOGY: I commented that tissues from this calf tested positive for Brucella when in fact the bacteriology report stated otherwise. The bacteriology report is correct. Brucella was not isolated from this bison calf.


D. J. Marshall, BVSc, PhD/mmm

REASON FOR SUBMISSION: Bison calf abortion

LABORATORY DIAGNOSIS:
Bison calf abortion; Brucella abortus

COMMENT: Brucella cultures for this case were inconclusive due to Proteus overgrowth.

D. J. Marshall, BVSc, PhD\cto

Date In 05/04/2015

PATHOLOGY

Date Out: 05/11/2015

Released by: JM

GROSS PATHOLOGY: A bison calf (ID 5G07) was submitted for necropsy. Necropsy is performed at 10.30 am 4th May 2015. Calf is autolyzed and predated. Male calf had a crown rump length measurement of 91 cm. Abdominal organs are missing. Brain was not examined.

HISTOPATHOLOGY: Sections of brain, lung, skeletal muscle and thymus. Tissues are moderately autolyzed. Lung is not aerated and alveoli and airways contain quantities of meconium and squamous epithelial debris.

MORPHOLOGIC DIAGNOSIS:

Lung: Non-aeration; Intra-alveolar meconium and squamous epithelial debris and meconium

Date In: 05/04/2015

BACTERIOLOGY

Date Out: 05/11/2015

Released by: mh

MVDL Accession #:
8-397-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA/APHIS/VS

Date In: 05/04/2015

Date Out: 05/11/2015 Released by: mh

Brucella culture results inconclusive due to Proteus overgrowth.

CULTURES

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	3+	NA
	fetal lung	Brucella	Proteus overgrowth		NA

1+ to 4+ = rare colony to confluent growth

P = pure culture, M = mixed or partially contaminated culture

MVDL Accession #:
8-397-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA/APHIS/VS

Fees

Bacteriology Fee	\$ 45.00
Pathology/Histology Fee	\$ 35.75
Accession Total Fee	<u>\$ 80.75</u>

(This is not a bill. Do not make payment from this report.)

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Tuesday, June 30, 2015 5:55:43 AM
Attachments: [doc.pdf](#)

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Friday, June 19, 2015 3:29 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 8 June 15
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only
(SEE EXAMPLE and KEY on back Page 4)

OWNER:	APHIS, VS
ADDRESS:	
CITY/STATE/ZIP:	CORNWALL SPRINGS, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)	BQFS - Gonorrhea - Cattle Heath.

SUBMITTER'S SIGNATURE: *P. Ryan Clarke*
SUBMITTER'S NAME (PRINT): *P. Ryan Clarke*
ADDRESS: **(b) (6)**
CITY/STATE/ZIP: **(b) (6)**
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: *B. Frey, R. Clarke, J. Ryan*

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.

[illegible]

Laboratory Comments:

Laboratory Comments:
See attached report for
final classification per DBE.

FEE:

DATE RECEIVED: 6-8-15

CASE # 8-452-15

The MVDL is an accredited AAEP Laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Friday, June 19, 2015 10:44 AM
To: Fuentes, Antonio
Subject: Re: Final classif. Case No. 8-452-15

On 6/18/2015 4:01 PM, Fuentes, Antonio wrote:

Greetings (b) (6)

Would you give us a final classif. For this bison from GonaCon Std.?

Thanks.
Antonio

Hi Antonio: Case#8-452-15, GonaCon study ,Dr. Ryan Clarke: All six animals are classified Reactors based on

(b) (6)

Designated Brucellosis Epidemiologist

positive serological reactions. Regards

Frank

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Tuesday, June 30, 2015 5:55:38 AM
Attachments: [doc.pdf](#)

Hi!

I am forwarding the test charts for the buffalo you are getting. Another to follow....I will send a pic of the 1-27 when I leave if you could forward it to CO? I don't have them in my phone, and don't want to try to type that in.....while driving! HA!

Let me know if you won't be around...should have asked yesterday, but got really busy! Shocking!

Thanks,

Becky

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]

Sent: Friday, June 19, 2015 3:30 PM

To: Clarke, Patrick R. - APHIS; Rhyen, Jack C - APHIS; Frey, Rebecca K - APHIS

Subject: scan

KM-2560

[00:c0:ee:1e:d7:d6]

DATE BLED: 6/9/15

REASON FOR TEST

☒ DIAGNOSTIC

☐ REGULATORY

☐ QUARANTINE/IMPORTS

STATE OF MONTANA

DEPT. OF LIVESTOCK DIAGNOSTIC LABORATORY DIVISION

BOX 997 — BOZEMAN, MT 59771 — PHONE (406) 994-4885 — FAX (406) 994-6344

SEROLOGY REPORT

COMPLETE SHADED AREAS ONLY

DATE RECEIVED: 6-9-15

SPECIES: Bison

COUNTY: Park

PAGE 1 OF PAGES 2

OWNER: Bison Quarantine Facility - Gonacon

ADDRESS: Parker

COLEM SPRING ZIP

SUBMITTED BY: Beckley Freet

ADDRESS: (b) (6)

ZIP: (b) (6)

DESTINATION/QUARANTINE NO./REMARKS: - Bison Funds - Dr. Zakaria Bruceellor's

If required, phone/FAX results to:

CIRCLE TEST REQUIRED — If specific test and/or standard dilution required, so indicate						BRU	BT	ANA	PTB	IBR	BVD	BLV	LEPTOSPIROSIS - 8 SEROVARS	OTHER
TUBE NO.	IDENTIFICATION	AGE	SEX	BREED	OFF. VAC.	Finax	Bru	Bru	Bru	Bru	FPA	FPA	mp values	
1	Red 01	AD	F	Bism	MA	Sus.	Neg	Neg	Neg	Susp	19.8 mP		(Suspect)	
2	Red 04					Reactor	Neg	Neg	2 ⁺ (1:10)	Pos	47.6 mP			
3	Red 05					Neg	Neg	Neg	Neg	Neg	-2.3 mP			
4	Red 11					Neg	Neg	Neg	Neg	Neg	8.9 mP			
5	Red 19					Reactor	Pos	Pos	Neg	Pos	181.9 mP			
6	Red 27					Reactor	Pos	Pos	Neg	Pos	159.3 mP			
7	Red 28					Reactor	Pos	Pos	3 ⁺ (1:10)	Pos	119.6 mP			
8	Red 29					Reactor	Pos	Neg	Neg	Pos	24.9 mP			
9	Red 31					Neg	Neg	Neg	Neg	Neg	-0.6 mP			
10	3603	2y	F			Neg	Neg	Neg	Neg	Neg	0.9 mP			
No. Samples						15	15	15	15	15				
No. Seropositive						5								
No. Suspect						1								
No. Seronegative						9								
No. Undetermined						—								
Comments														
TESTED BY														

Please do FPA, CF, BAPA & Card.

email: Patrick Rym Clarke
+ Rebecca Froy
Released 6-19-15

STATE OF MONTANA

DEPT. OF LIVESTOCK DIAGNOSTIC LABORATORY DIVISION
BOX 997 — BOZEMAN, MT 59771 — PHONE (406) 994-4885 — FAX (406) 994-6344

CONTINUATION SEROLOGY REPORT

COMPLETE SHADED AREAS ONLY

OWNER	Bison Aquaculture - Genaleen
SUBMITTED BY	R. Frey
DATE	6/9/15
PAGE	2 OF 2

Brucellosis

CIRCLE TEST REQUIRED — If specific test and/or standard dilution required, so indicate						BRU	BT	ANA	PTB	IBR	BVD	BLV	LEPTOSPIROSIS - 8 SEROVARS								OTHER
TUBE NO.	IDENTIFICATION	AGE	SEX	BREED	OFF. VAC.	Final	Brn	Brn	Brn	Brn	PPA	6/18/15									
							Brn	Brn	Brn	Brn	mp values										
11	3617	2	F	Brn	u/a	Neg	Neg	Neg	Neg	Neg	-0.5 mP										
12	4602	1	M			Neg	Neg	Neg	Neg	Neg	0.7 mP										
13	4606	1	M			Neg	Neg	Neg	Neg	Neg	3.7 mP										
14	4617	1	F			Neg	Neg	Neg	Neg	Neg	1.3 mP										
15	4702	1	M			Neg	Neg	Neg	Neg	Neg	0.8 mP										

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Thursday, June 18, 2015 11:31 AM
To: Fuentes, Antonio
Subject: Re: Final bru classif.

On 6/18/2015 10:50 AM, Fuentes, Antonio wrote:

Greetings (b) (6)

Hope you are enjoying this nice weather.

Would you be able to give us a final classification for two GonaCon Study bison charts.
Thanks.

Have a good day,
Antonio

Hi Antonio: Gona Con Study Dr. Ryan Clarke Case#8-439-15. The following are classified as reactors based on positive serological reactions.
Tubes#s 2 R45,#3 R39,#4 R47,#6 R54,#7 R48, #12 R38,,#15 R53, #17R42,#17 R42,#18 R41,#19 R56,#20 R34,#25 R44, #26 R36, #27, R55, #28 R43, #29 R49,#30 R46, R51
Tubes#s 13 Gr 19 & #14 GR 29 are classified as suspects.

Case#8-455-15, Tubes#s 2 R04, #5 R19, #6 R27, #7 R28, #8 R29 are classified as reactors. Tube# 1 R01 is classified as a suspect.

Regards Frank

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Tuesday, June 30, 2015 5:52:04 AM
Attachments: [doc.pdf](#)

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Friday, June 19, 2015 3:30 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

DATE BLED: 6/9/15

REASON FOR TEST

☒ DIAGNOSTIC

☐ REGULATORY

☐ QUARANTINE/IMPORTS

STATE OF MONTANA

DEPT. OF LIVESTOCK DIAGNOSTIC LABORATORY DIVISION

BOX 997 — BOZEMAN, MT 59771 — PHONE (406) 994-4885 — FAX (406) 994-6344

SEROLOGY REPORT

COMPLETE SHADED AREAS ONLY

DATE RECEIVED: 6-9-15

SPECIES: Bison

COUNTY: Park

PAGE 1 OF PAGES 2

OWNER: Bison Quarantine Facility - Gonacon

ADDRESS: Parker

COLOWM Springs

ZIP

SUBMITTED BY: Becklin Freet

ADDRESS: (b) (6)

ZIP: (b) (6)

DESTINATION/QUARANTINE NO./REMARKS: - Bison Funds - Dr. Zakaria Bruceellor's

If required, phone/FAX results to:

CIRCLE TEST REQUIRED — If specific test and/or standard dilution required, so indicate						BRU	BT	ANA	PTB	IBR	BVD	BLV	LEPTOSPIROSIS - 8 SEROVARS	OTHER
TUBE NO.	IDENTIFICATION	AGE	SEX	BREED	OFF. VAC.	Finax	Bru	Bru	Bru	Bru	FPA	FPA	mp values	
1	Red 01	AD	F	Bism	MA	Sus.	Neg	Neg	Neg	Susp	19.8 mP		(Suspect)	
2	Red 04					Reactor	Neg	Neg	2 ⁺ (1:10)	Pos	47.6 mP			
3	Red 05					Neg	Neg	Neg	Neg	Neg	-2.3 mP			
4	Red 11					Neg	Neg	Neg	Neg	Neg	8.9 mP			
5	Red 19					Reactor	Pos	Pos	Neg	Pos	181.9 mP			
6	Red 27					Reactor	Pos	Pos	Neg	Pos	159.3 mP			
7	Red 28					Reactor	Pos	Pos	3 ⁺ (1:10)	Pos	119.6 mP			
8	Red 29					Reactor	Pos	Neg	Neg	Pos	24.9 mP			
9	Red 31					Neg	Neg	Neg	Neg	Neg	-0.6 mP			
10	3603	2y	F			Neg	Neg	Neg	Neg	Neg	0.9 mP			
No. Samples						15	15	15	15	15				
No. Seropositive						5								
No. Suspect						1								
No. Seronegative						9								
No. Undetermined						—								
Comments														
TESTED BY														

2 attached documentation from test

Please do FPA, CF, BAPA & Card.

email: Patrick Rym Clarke & Rebecca Fry

Released 6-19-15

DEPT. OF LIVESTOCK DIAGNOSTIC LABORATORY DIVISION
BOX 997 — BOZEMAN, MT 59771 — PHONE (406) 994-4885 — FAX (406) 994-6344

COMPLETE SHADED AREAS ONLY

OWNER	Bison Acquisition - Gonaheim
SUBMITTED BY	R. Frey
DATE	6/9/15
PAGE	2 OF 2

[illegible]

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Thursday, June 18, 2015 11:31 AM
To: Fuentes, Antonio
Subject: Re: Final bru classif.

On 6/18/2015 10:50 AM, Fuentes, Antonio wrote:

Greetings (b) (6)

Hope you are enjoying this nice weather.

Would you be able to give us a final classification for two GonaCon Study bison charts.
Thanks.

Have a good day,
Antonio

Hi Antonio: Gona Con Study Dr. Ryan Clarke Case#8-439-15. The following are classified as reactors based on positive serological reactions.
Tubes#s 2 R45,#3 R39,#4 R47,#6 R54,#7 R48, #12 R38,,#15 R53, #17R42,#17 R42,#18 R41,#19 R56,#20 R34,#25 R44, #26 R36, #27, R55, #28 R43, #29 R49,#30 R46, R51
Tubes#s 13 Gr 19 & #14 GR 29 are classified as suspects.

Case#8-455-15, Tubes#s 2 R04, #5 R19, #6 R27, #7 R28, #8 R29 are classified as reactors. Tube# 1 R01 is classified as a suspect.

Regards Frank

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Tuesday, June 30, 2015 5:52:01 AM
Attachments: [doc.pdf](#)

Here are the test charts for the bison coming tomorrow. The other is in the next email.

I will send you a pic of the 1-27 when I leave if you can forward to all of the CO folks. I just don't have them in my phone, and I can't be typing them in while driving! Is that ok?

Thanks

Becky

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]

Sent: Friday, June 19, 2015 3:29 PM

To: Clarke, Patrick R. - APHIS; Rhyen, Jack C - APHIS; Frey, Rebecca K - APHIS

Subject: scan

KM-2560

[00:c0:ee:1e:d7:d6]

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 8 June 15
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only
(SEE EXAMPLE and KEY on back Page 4)

OWNER: *APHIS, VS*
ADDRESS:
CITY/STATE/ZIP: *CORWIN SPRINGS, MT*
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS - Gon & Con - Gattle Heath.

SUBMITTER'S SIGNATURE: *P. Ryan Clarke*
SUBMITTER'S NAME (PRINT): *P. Ryan Clarke*
ADDRESS: **(b) (6)**
CITY/STATE/ZIP: **(b) (6)**
RESULT REPORTING OPTIONS: ☐ PHONE / ☐ FAX / ☐ EMAIL
NUMBER OR EMAIL ADDRESS: *B. Frey, R. Clarke, J. Ryan*

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.

[illegible]

Laboratory Comments:

See attached report for final classification per DBE.

FEE:

DATE RECEIVED: 6-8-15

CASE # 8-452-15

The MVDL is an accredited AAEP Laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

additionally

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Friday, June 19, 2015 10:44 AM
To: Fuentes, Antonio
Subject: Re: Final classif. Case No. 8-452-15

On 6/18/2015 4:01 PM, Fuentes, Antonio wrote:

Greetings (b) (6)

Would you give us a final classif. For this bison from GonaCon Std.?

Thanks.
Antonio

Hi Antonio: Case#8-452-15, GonaCon study ,Dr. Ryan Clarke: All six animals are classified Reactors based on

(b) (6)

Designated Brucellosis Epidemiologist

positive serological reactions. Regards

Frank

From: [Rhyen, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Monday, June 01, 2015 9:42:43 AM
Attachments: [doc.pdf](#)

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Thursday, May 28, 2015 4:05 PM
To: Clarke, Patrick R. - APHIS; Rhyen, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

The MVDL is an accredited AAEP laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

Fuentes, Antonio

From: (b) (6)@gmail.com>
Sent: Thursday, May 28, 2015 1:59 PM
To: Fuentes, Antonio
Subject: Re: Final Bru. Classification

On 5/28/2015 1:20 PM, Fuentes, Antonio wrote:

Greetings (b) (6)

See attached chart for final brucellosis classification.
Thanks.

Have a good day,
Antonio

Hi Antonio: GonaCon study case#8-403-15 Dr. Ryan Clarke. ID# R24 is classified as a reactor based on FP results. 5R07 (calf) is classified as a reactor based on CF results(this CF reaction may be a non specific

(b) (6)

Designated Brucellosis Epidemiologist

background reaction) Regards Frank

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Monday, May 18, 2015 4:27:18 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, May 18, 2015 11:45 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

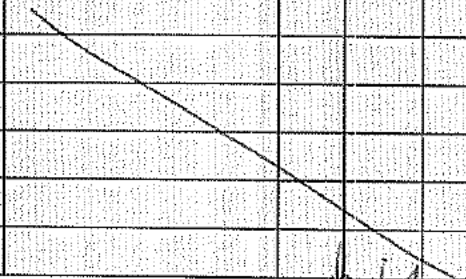
Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 5-5-15
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS-US
ADDRESS:
CITY/STATE/ZIP: Corwin Springs, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS - Bona Con - Cattle Health

SUBMITTER'S SIGNATURE: [Signature]
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS: (b) (6)
CITY/STATE/ZIP:
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: B. Frey, R. Clarke, J. Rhyon

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	EHD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.										
1	G07	Ad	Fe	Bison		Neg	N	N	N	N (0.4)					
2	R09					Reactor	Pos	Pos	4/160	Pos (179.7)					
3	R02					Neg	N	N	N	N (3.8)					
4	5R02	calf				Neg	N	N	3/20	N (-59.1)					
															
Laboratory Comments: <u>Please do FPA, CF Card, BAPA</u>						Samples	4	4	4	4	4				
<u>See attached report from Dr. Roark (DBE)</u>						Seropositive	1								
						Suspect									
						Seronegative	3								
Released <u>ap 5/18/15</u>						Undetermined									
						Tested By	<u>5/18/15</u>								

FEE: _____

DATE RECEIVED: 5-5-15

CASE # 8-401-15

The MVDL is an accredited AAEP Laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 1/09)

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Monday, May 18, 2015 10:36 AM
To: Fuentes, Antonio
Subject: Re: Final Bru. classification

On 5/18/2015 8:32 AM, Fuentes, Antonio wrote:

Greetings (b) (6)

Need a final classification for these four bison from the GonaCon study.
Thanks.

Have a good day,
Antonio

Hi again Antonio: Case# 8-401-15, Dr.Ryan Clarke, Gona Con Study
Tube# 2 ID R09 is classified as a reactor based on positive serological reactions. Regards Frank

(b) (6)

Designated Brucellosis Epidemiologist

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Wednesday, April 08, 2015 4:17:18 PM
Attachments: [doc.pdf](#)

FYI

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]

Sent: Wednesday, April 08, 2015 4:33 PM

To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS

Subject: scan

KM-2560

[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406) 994-4885
Fax: (406) 994-6344
Email: livdiagnosticlab@mt.gov

Accession # 8-336-15

Owner: GONA CON STUDY

Submitter: PATRICK RYAN CLARKE D.V.M.
187 TOBIANO TRAIL
BELGRADE MT 59714

Species: WILD - BISON

Breed: NA

Name/No. 5G14

Age: FETUS Sex:

Date Sent: 04/08/2015

Date Received: 03/20/2015

Final Report

Case Coordinator: JM

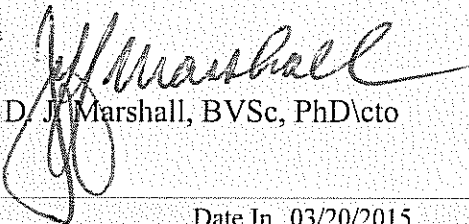
CASE SUMMARY

REASON FOR SUBMISSION: Bison abortion

LABORATORY DIAGNOSIS:

Bison abortion; Placentitis; Brucella abortus culture positive

COMMENT: Brucella abortus was cultured from abomasal contents and placenta. This isolate was forwarded to NVSL and confirmed as being B abortus (see attached report).


D. J. Marshall, BVSc, PhD

Date In 03/20/2015

PATHOLOGY

Date Out: 04/08/2015

Released by: JM

GROSS PATHOLOGY: A bison fetus and piece of placenta were submitted for necropsy and subsequent laboratory evaluation. Necropsy was performed at 12.00 pm on Friday 20th March 2015. Female fetus had a crown rump measurement of 64 cm and was in a good state of post mortem preservation. No significant gross abnormalities are detected.

HISTOPATHOLOGY: Sections of placenta, brain, liver, kidney, heart, lung, spleen abomasum, small intestine and skeletal muscle are examined. Placenta is multifocally inflamed and necrotic. Lung is not aerated and small quantities of squamous epithelial debris and macrophages are present in alveoli. No significant histological abnormalities are present in the remaining tissues.

MORPHOLOGIC DIAGNOSIS:

Placenta: Necrotizing placentitis, multifocal

Lung: Non-aeration; Alveolitis with intra-alveolar squamous epithelial debris

Date In 03/20/2015

BACTERIOLOGY

Date Out: 04/01/2015

Released by: mh

Isolate sent to NVSL 3/25/15 for species ID and genotyping.

CULTURES

ID/Site	Specimen	Culture Type	Isolate	Antimicrobial	
				Growth	Profile
	abomasal contents	Aerobic	Brucella abortus	4+ P	NA
	abomasal contents	Brucella	Brucella abortus	4+ P	NA
	abomasal contents	Campylobacter	Negative for Campylobacter sp.		NA
	placenta	Aerobic	Brucella abortus	4+ M	NA
	placenta	Brucella	Brucella abortus	4+ M	NA

002641

MVDL Accession #
B-336-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
GONA CON STUDY

Date In 03/20/2015

Date Out: 04/01/2015 Released by: mh

placenta

Campylobacter Negative for Campylobacter sp.

NA

1+ to 4+ = rare colony to confluent growth

P = pure culture, M = mixed or partially contaminated culture

Date In: 03/25/2015

REFERRAL/OTHER

Date Out: 04/01/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrl Inst.</u>
5G14	Slant tube	Brucella Culture	Brucella abortus.	NVSL

Please see attached report for complete results.



National Veterinary Services Laboratories

FINAL REPORT

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner
Gona Con Study
Corwin Springs, MT

Accession Number: 15-009841

Animal Location

Park County MT

Date Collected: 03/20/2015

Date Received: 03/26/2015

Submitter - 2046
MT Department of Livestock
Diagnostic Laboratory Division
1911 W Lincoln St
PO Box 997
Bozeman, MT 59718
FAX #: 406-994-6344
Phone #: 406-994-4885

Date Completed: 04/01/2015

Collected By: Dr. P. Ryan Clarke

Purpose: General Diagnostic

Referral Number: 8-336-15

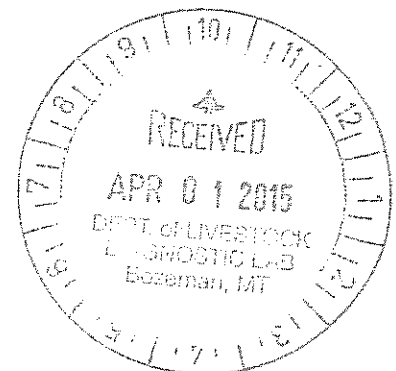
This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 8-336-15 Animal ID: 5G14 Brucella Case Number: B15-0106 Specimen Type: Culture Species: Bison

Brucella Final Identification

Brucella abortus



Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Scanned 4-1-15/jm
cc: Bact- 4-1-15/jm

MVDL Accession #
8-336-15

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
GONA CON STUDY

Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 73.50
Referral Fee	\$ 19.10
Accession Total Fee	\$ 92.60

(This is not a bill. Do not make payment from this report.)

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Friday, February 06, 2015 11:51:22 AM
Attachments: [doc.pdf](#)

July test of Red 421.....see 81AJW3758

Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Friday, July 25, 2014 3:35 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Dark

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 7-15-14
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS VS
ADDRESS:
CITY/STATE/ZIP: Cornwall Springs, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS-GonaCon - Cattle Health

SUBMITTER'S SIGNATURE: P. Ryan Clarke
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS:
CITY/STATE/ZIP: (b) (6)
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: R. Clarke, B. Fray, Jack Ryan

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.

TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	BRU	BT	ANA	EHD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
1	Grn 27	Ad	Fe	Bison	N/A	Neg	N	N	N ₂₅ /+50	N	N	0.3			
2	Grn 30								N ₂₅ /+50			0.2			
3	Grn 26								N ₂₅ /+100			0.7			
4	Grn 25								N ₂₅ /+50			~0.3			
5	Grn 21								N ₂₅ /+50			0.0			
6	Grn 24								N ₂₅			0.8			
7	Grn 22								N ₂₅ /+100			~0.8			
8	Grn 23								N ₂₅ /+50			1.4			
9	Grn 20								+50			7.1			
10	BIATW 3758					R	P ₂₅		N ₂₅ /+100	4:20		8.2			
R → Deceptor						Samples	10	10	10	10	10				
Laboratory Comments:						Seropositive	1								
						Suspect									
						Seronegative	9								
						Undetermined									
						Tested By	7/25/14								

Please Do BAPA, CF
RIV, Cord, FPA
* See attach report for final classification per DBE.

Released 7/25/14

FEE:

DATE RECEIVED:

7/15/14

CASE #

9-26-15-10
8-45-15

The MVDL is an accredited AAVID laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

1/28

Fuentes, Antonio

From: (b) (6) <[REDACTED]@gmail.com>
Sent: Friday, July 25, 2014 11:49 AM
To: Fuentes, Antonio; Horak, Sarah; Knopp, Doug; Clarke,Patrick
Subject: GonaCon study results

Case#8-45--15 Tube#10 81AJW3758 is classified as a reactor. The rest are negative.
Case#8-37-15 Tubes #1,2&3 Red60,Red,58,Red57 are classified reactors the rest are negative.
Case#8-40-15 Tube#6 YNP930797 is classified as a reactor , the rest are negative

(b) (6)

Designated Brucellosis Epidemiologist

Regards and thanks Antonio Frank



This email is free from viruses and malware because avast! Antivirus protection is active.

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Friday, February 06, 2015 11:50:06 AM
Attachments: [doc.pdf](#)

Oct test of Red 421.....see 81AJW3758

Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, November 10, 2014 3:24 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771

Phone (406) 994 - 4885 Fax (406) 994 - 6344

Email: livdiagnosticlab@mt.gov

Collection Date: 10/30/14
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS, VS
ADDRESS:
CITY/STATE/ZIP: COTWIN SPRINGS, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQ FS - Gona Con - Cattle Health

SUBMITTER'S SIGNATURE: [Signature]
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS: (b) (6)
CITY/STATE/ZIP:
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: J. Rhymer, B. Frey, R. Clarke

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	EHD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	Final classif.	Brucella FPA	Brucella BAPA	Brucella CF	Brucella Card		ap 11/10/14		8 - SEROVARs	
1	B1 AJW 3758	Ad	Fe	Bison		Neg	Neg	N	Neg	N					
2	Gr 20						Neg		Neg						
3	Gr 22						Neg		Neg						
4	Gr 23						Neg		Neg						
5	Gr 26						Neg		Neg						
6	Gr 27						Neg		Neg						
7	Gr 30						Neg		Neg						
Laboratory Comments:						Samples	7	7	7	7	7				
Please do FPA, CF						Seropositive									
BAPA, Card, CF						Suspect									
						Seronegative	7								
						Undetermined			CF (1:10)						
						Tested By	11-10-14		ap 11/10/14						

Released ap 11-10-14

FEE:

DATE RECEIVED: 10-31-14

CASE # 8-239-15

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Friday, February 06, 2015 11:45:03 AM
Attachments: [doc.pdf](#)

This has test result for Red 421.

Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, February 02, 2015 3:41 PM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 1-14-15
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only
(SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS, VS
ADDRESS:
CITY/STATE/ZIP: Catwin Springs, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS - Gonadon - Cattle Health

SUBMITTER'S SIGNATURE: [Signature]
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS: (b) (6)
CITY/STATE/ZIP: (b) (6)
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: R. Clarke, B. Fray, J. Rhyam

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	END	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	BRU	BT	ANA	END	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
1	R 421	calf		Bison	* Suspect	Final	Ben	Ben	Ben	Ben					
2	364					Reactor	Pos	Pos	3+ (1:160)	P (226.4)					
3	3 R22					Reactor	Pos	Pos	3+ (1:10)	P (212.6)					
4	3 GDB					Reactor	Pos	Pos	3+ (1:320)	P (189.2)					
5	3 R20					Reactor	Pos	N	(1:20)	P (49.4)					
See attach document from DBE for final Laboratory Comments: <u>Classification 2/11/30/15</u>						Samples	5	5	5	5	5	(FPA Delta m value)			
Please do FPA, BAPA						Seropositive									
CF, Card,						Suspect									
* Correction: Tube #1 (R 421)						Seronegative									
is classified as "SUSPECT" 2/11/30/15						Undetermined									
						Tested By									

FEE: _____

DATE RECEIVED: 1-16-15

CASE # 8-277-15

The MVDL is an accredited AAVLD laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

Fuentes, Antonio

From: (b) (6) gmail.com>
Sent: Friday, January 30, 2015 11:58 AM
To: Fuentes, Antonio; Horak, Sarah; Knopp, Doug; Liska, Eric; Linfield, Tom; Zaluski, Martin; Thompson, Brent; Clarke, Patrick
Subject: GonaCon Bison Study Case#8-277-15

Tubes#s 2 3G4,3 3R22,4 3G08 and 5 R20 are classified as Reactors Tube#1 R47 is classified as a

(b) (6)

Designated Brucellosis Epidemiologist

suspect

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Monday, November 10, 2014 3:20:17 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, November 10, 2014 3:24 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771

Phone (406) 994 - 4885 Fax (406) 994 - 6344

Email: livdiagnosticlab@mt.gov

Collection Date: 10/30/14
Page 1 of 1

Collection Date: 10/30/14
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only
(SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS, VS
ADDRESS:
CITY/STATE/ZIP: Corwin Springs, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS - GonaCon - Cattle Heaty

SUBMITTER'S SIGNATURE:

SUBMITTER'S NAME (PRINT):

ADDRESS:

CITY/STATE/ZIP:

RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL

NUMBER OR EMAIL ADDRESS: J. Rhyon, B. Frey, K. Clarke

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	EHD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	Final Classif.	FPA	BAPA	CF (1:10)	Card					
1	Bl AJW 3758	Ad	Fe	B/Ben		Neg	Neg	N	Neg	N					
2	Gr 20						Neg		Neg						
3	Gr 22						Neg		Neg						
4	Gr 23						Neg		Neg						
5	Gr 26						Neg		Neg						
6	Gr 27						Neg		Neg						
7	Gr 30					✓	Neg	✓	Neg	✓					
						Samples	7	7	7	7	7				
						Seropositive									
						Suspect									
						Seronegative	7								
						Undetermined				CF (1:10)					
						Tested By	AP			ap					

Laboratory Comments:

Please do FPA, CF
BAPA, Card, ~~Box~~

Released ap 11-10-14

FEE:

DATE RECEIVED: 10-31-14

CASE # 8-239-15

The MVDL is an accredited AAHL laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Wednesday, June 18, 2014 10:47:33 AM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, June 18, 2014 8:38 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: scan

Jack, what do you think?

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, June 16, 2014 4:22 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406) 994-4885
Fax: (406) 994-6344
Email: livdiagnosticlab@mt.gov

Accession #: 8-404-14

Owner: USDA, APHIS, VS

Species: WILD - BISON

Breed: BISON

Name/No. 4G10

Age: NEWB(Sex:

Date Sent: 06/16/2014

Date Received: 06/04/2014

Submitter: PATRICK RYAN CLARKE D.V.M.

187 TOBIANO TRAIL

BELGRADE MT 59714

Final Report

Case Coordinator: AWL

CASE SUMMARY

REASON FOR SUBMISSION: Abortion; Brucella abortus seropositive

LABORATORY DIAGNOSIS:

Bison abortion; etiology Streptococcus uberis

COMMENT: Heavy pure growth of Streptococcus uberis was isolated from the abomasal contents. Brucella and Campylobacter cultures were negative. The animal is classified as a Brucella reactor based on serologic results. See attached for results.

A. W. Layton, DVM, DACVP\rb

Date In 06/04/2014

PATHOLOGY

Date Out: 06/16/2014

Released by: AWL

GROSS: The carcass is of a female, 60 cm crown/rump length, fully haired bison fetus in poor to fair post mortem and good nutritional state. Abomasum contains thick pink fluid. Lungs are collapsed.

HISTOPATHOLOGY: Tissue sections of kidney, adrenal gland, ileum, abomasum, spleen, lung, heart, liver, spinal cord, diaphragm and thymus are examined. Alveolar spaces are collapsed and many spaces contain sloughed epithelial cells, few macrophages, occasional neutrophils, squamous epithelial cells and meconium. Endothelium of veins within the liver have variable degrees of mineralization.

MORPHOLOGIC DIAGNOSIS:

Pneumonia, mild with atelectasis

Meconium and squamous inhalation

Autolysis, moderate

Date In: 06/05/2014

BACTERIOLOGY

Date Out: 06/13/2014

Released by: jl

CULTURES

ID/Site	Specimen	Culture Type	Isolate	Growth	Antimicrobial Profile
	abomasal contents	Campylobacter	Negative for Campylobacter sp.		NA
	abomasal contents	Aerobic	Streptococcus uberis	4+ P	NA
	abomasal contents	Brucella	Negative for Brucella sp.		NA

1+ to 4+ = rare colony to confluent growth

P = pure culture, M = mixed or partially contaminated culture

Date In: 06/04/14

SEROLOGY

Date Out: 06/13/14

Released by: AF

002656

MVDL Accession #:
8-404-14

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA, APHIS, VS

SEROLOGY

Test Summary

Testname	# of tests	# Negative	# Positive	# Suspect	# A_C	# Undetermined	# Insufficient	Tech
B. ABORTUS CF	1	0	1	0	0	0	0	AF
B. ABORTUS FP	1	0	1	0	0	0	0	DK
B. ABORTUS BAPA	1	0	1	0	0	0	0	DK
B. ABORTUS CARD	1	0	1	0	0	0	0	DK
B. ABORTUS RIVANOL	1	0	1	0	0	0	0	DK

List of Significant results

Animal Id	Testname	Result	Titer
GR10	B. ABORTUS CF	POS	4+160
GR10	B. ABORTUS RIVANOL	POS	+200
GR10	B. ABORTUS CARD	POS	
GR10	B. ABORTUS BAPA	POS	
GR10	B. ABORTUS FP	POS	159.7

Final Classification

Animal Id	Classification	Comment
GR10	REACTOR	Classified by Dr. Houle (DBE)

5/25/42

Knopp, Doug

From: (b) (6) gmail.com>
Sent: Wednesday, June 11, 2014 11:30 AM
To: Knopp, Doug
Subject: Re: Classification of Bison 8-404-14

On 6/11/2014 9:34 AM, Knopp, Doug wrote:

Hi (b) (6)
Here is another bison to classify. It should be pretty

(b) (6)

Designated Brucellosis Epidemiologist

easy.

Doug

Good Morning Doug: Case#8-404-14 Dr. Ryan Clarke Adult Female Bison
Tube#1 Gr. 10 Is classified as a reactor



This email is free from viruses and malware because avast! Antivirus protection is active.

MVDL Accession #:
8-404-14

Submitter:
PATRICK RYAN CLARKE D.V.M.

Owner:
USDA, APHIS, VS

Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 70.00
Serology Fee	\$ 9.50
Accession Total Fee	\$ 79.50

(This is not a bill. Do not make payment from this report.)

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: scan
Date: Friday, July 26, 2013 4:20:02 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Frey, Rebecca K - APHIS
Sent: Friday, July 26, 2013 9:31 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

Curious. These are the non-pregnant animals from the treatment group.
Also, do you remember the name of the vet clinic we traded bulls at the last time we met in Buffalo?
Thanks

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Wednesday, July 24, 2013 11:05 AM
To: Patrick.R.Clarke@aphis.gov; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771
Phone (406) 994 - 4885 Fax (406) 994 - 6344
Email: livdiagnosticlab@mt.gov

Collection Date: 7/11/13
Page 1 of 2

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA-APHIS-VS - Gona Con
ADDRESS:
CITY/STATE/ZIP: Carwin Springs, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS - Gona Con

SUBMITTER'S SIGNATURE: [Signature]
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS:
CITY/STATE/ZIP: (b) (6)
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: J. Ryan, R. Clarke, B. Frey

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BD	APA	EMD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	BAPA	Brd	Brd	Brd	Brd					
1	Red 29	Ad	Fe	Bison		Pos	Pos	ISO	3+80	54.5	15.6				
2	Red 28					Pos	Pos	4200	N	Pos	200.4				
3	Green 04					N	N	N	N	N	3.6				
4	Red 02					Pos	Pos	4200	2+10	Pos	172.7				
5	Red 23					Pos	Pos	N	N	Pos	68.5				
6	Red 14					Pos	Pos	4200	4+80	Pos	154.4				
7	Green 06					Pos	N	N	2+10	Pos	30.5				
8	Red 04					N	N	N	N	N	4.5				
9	Red 11					N	N	N	N	N	4.0				
10	Red 27					N	N	N	N	N	6.0				
Laboratory Comments:						Samples	14	14	14	14	14				
						Seropositive	8	6	4	3	8				
						Suspect			1	2	1				
						Seronegative	6	8	9	9	5				
						Undetermined									
						Tested By	<u>DK</u>								

BAPA, FPA, Card, CF, RIV
please

FEE: _____ DATE RECEIVED: 7-11-13 CASE # 8-27

Species: Bison
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771

Phone (406) 994 - 4885 Fax (406) 994 - 6344

Email: livdiagnosticlab@mt.gov

Collection Date: 7/11/13
Page 2 of 2

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA-APHIS-VS-Gona Con
ADDRESS:
CITY/STATE/ZIP: CORWIN SPRINGS, MT
REASON FOR TEST - MANDATORY INFORMATION (See Key)
BQFS-Gona Con

SUBMITTER'S SIGNATURE: [Signature]
SUBMITTER'S NAME (PRINT): P. Ryan Clarke
ADDRESS:
CITY/STATE/ZIP: (b) (6)
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL
NUMBER OR EMAIL ADDRESS: J. Phylon, R. Clarke, B. Fray

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	EH	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	BAPA	Card	RIV	CF	FP					
11	Red 01	Ad	Fe	Bison		N	N	N	N	N	9.6				
12	Red 31	↓	↓	↓		N	N	N	N	Pos	46.5				
13	Red 05	↓	↓	↓		Pos	N	N	240	Pos	64.5				
14	Red 19	↓	↓	↓		Pos	Pos	F200	N	Pos	157.8				
Laboratory Comments:						Samples									
						Seropositive									
						Suspect									
						Seronegative									
						Undetermined									
						Tested By									

Laboratory Comments:

BAPA, Card, FPA, CF, RIV

FEE: _____ DATE RECEIVED: _____ CASE # 8-27

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: scan
Date: Thursday, July 16, 2015 2:43:07 PM
Attachments: [doc.pdf](#)

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Thursday, July 16, 2015 10:36 AM
To: Rhyan, Jack C - APHIS
Subject: FW: scan

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Friday, May 15, 2015 4:20 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]



MVDL

MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771
1911 West Lincoln Street Bozeman, MT 59718
Website: www.liv.mt.gov/lab

Phone: (406) 994-4885
Fax: (406) 994-6344
Email: livdiagnosticlab@mt.gov

Accession # 8-396-15
Owner: USDA/APHIS/VS

Species: WILD - BISON
Breed: BISON
Name/No. 5R09
Age: NEWB(Sex:

Date Sent: 05/15/2015
Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.
187 TOBIANO TRAIL
BELGRADE MT 59714

Final Report

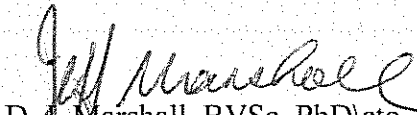
Case Coordinator: JM

CASE SUMMARY

5/15/15

ADDITIONAL INFORMATION:

BACTERIOLOGY: The Brucella isolate was confirmed as Brucella abortus by identification testing at NVSL (see attached report).


D. J. Marshall, BVSc, PhD\cto

ADDITIONAL INFORMATION 5/13/15:

BACTERIOLOGY: Brucella sp was isolated from this calf. The isolate has been forwarded to NVSL for further identification procedures. Results will be forwarded as soon as available.

D. J. Marshall, BVSc, PhD/mmm

5/11/15

REASON FOR SUBMISSION: Bison calf abortion

LABORATORY DIAGNOSIS:

Bison calf abortion

COMMENT: Results of bacteriological investigations will be reported as soon as complete.

D. J. Marshall, BVSc, PhD\cto

Date In 05/04/2015

PATHOLOGY

Date Out: 05/11/2015

Released by: JM

GROSS PATHOLOGY: A bison calf (ID 5R09) was submitted for necropsy. Necropsy is performed at 11 am 4th May 2015. Calf is autolyzed and predated. Sex could not be determined. Crown rump length measured 76 cm. Only a small portion of brain, lung and skeletal muscle was available for examination. Brain was severely autolyzed and not sampled.

HISTOPATHOLOGY: Sections of lung and skeletal muscle are examined. Lung is severely autolyzed and not useful for diagnostic purposes. No significant abnormality is detected in skeletal muscle.

MORPHOLOGIC DIAGNOSIS:

Lung: Autolysis

Date In 05/04/2015

BACTERIOLOGY

Date Out: 05/15/2015

Released by: mh

Isolate to be sent to NVSL for full identification 5/12/15.

CULTURES

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	2+	NA
	fetal lung	Brucella	Brucella abortus	3+ M	NA

1+ to 4+ = rare colony to confluent growth

! = pure culture, M = mixed or partially contaminated culture

Date In: 05/11/2015

REFERRAL/OTHER

Date Out: 05/15/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
5R09	Slant Tube	Brucella Culture	See attached report	NVSL

Please see attached report for complete results.



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA, APHIS, VS
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 2047

MT Department of Livestock
Diagnostic Laboratory Division
1911 W Lincoln St
PO Box 997
Bozeman, MT 59718
FAX #: 406-994-6344
Phone #: 406-994-4885

Accession Number:

15-015494

Date Collected:

05/02/2015

Date Received:

05/13/2015

Date Completed:

05/15/2015

Collected By:

Dr. Patrick Ryan Clarke

Purpose:

General Diagnostic

Referral Number:

8-396-15

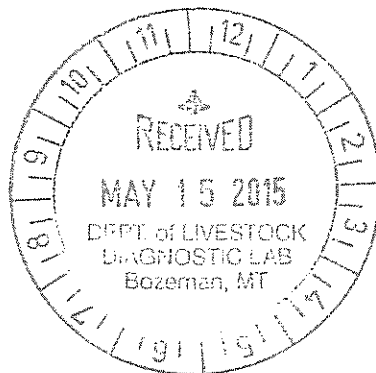
This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 8-396-15 Animal ID: 5R09 Brucella Case Number: B15-0160 Specimen Type: Culture Species: Bison

Brucella Final Identification

Brucella abortus



Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Scanned 5-15-15

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 73.50
Referral Fee	\$ 19.10
Accession Total Fee	\$ 92.60

(This is not a bill. Do not make payment from this report.)

From: [Jack C Rhyan](#)
To: [Matt McCollum](#); [Pauline Nol](#)
Subject: FW: Select agent information
Date: Tuesday, May 10, 2011 4:56:00 PM

-----Original Message-----

From: Rick_Wallen@nps.gov [mailto:Rick_Wallen@nps.gov]
Sent: Tuesday, May 10, 2011 4:22 PM
To: Rhyan, Jack C (APHIS)
Cc: Clarke, Ryan P. (APHIS); Jenny_Powers@nps.gov; Margaret_Wild@nps.gov; Frey, Rebecca K (APHIS)
Subject: Fw: Select agent information

Jack, I have deleted the many emails with the whole group of folks in this conversation so please pass along to Pauline and Matt.

Below is the note I received back from Ms. Barrow in CDC regarding how to interpret the overlap select agents regulations. I suspect the two individuals she included on cc are supervisors and also good contacts for further clarification on the interpretation of "naturally occurring environment".

Good discussion today. RW

----- Forwarded by Rick Wallen/YELL/NPS on 05/10/2011 03:11 PM -----

"Barrow, Yvonne
(CDC/OPHPR/DSAT)"
<iic8@cdc.gov> To
<rick_wallen@nps.gov>
05/06/2011 03:36 cc
PM "Harris, Lazenia (CDC/OPHPR/DSAT)"
<cw3@CDC.GOV>, "Miller, Yoon
(CDC/OPHPR/DSAT)" <eeu9@CDC.GOV>
Subject
Select agent information

Good afternoon,

Per our earlier conversation I have spoken with my supervisor and I could not find the e-mail that contained the actual guidance. I will try to locate that information for you as soon as possible. However, I did find a citation in our regulations that may help you. The select agent and toxin citation is 42 CFR 73.4(d)1:

(d) Overlap select agents or toxins that meet any of the following criteria are excluded from the requirements of this part:

(1) Any overlap select agent or toxin that is in its naturally occurring environment provided that the select agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.

This basically means as long as the select agent is in its naturally occurring environment (the bison) it is not considered a select agent. I hope this helps. Again I will try to look for the official e-mail that went out to our entities in regards to your question. If you need any other help please let me know.

Thanks,

Yvonne

Yvonne Barrow, M.S.
Microbiologist

Division of Select Agents and Toxins

Office of Public Health Preparedness and Response

Centers for Disease Control and Prevention
1600 Clifton Road, NE, MS A-46
Atlanta, GA 30333
Ph.: 404-718-2059 Fax: 404-718-2096 cell: (b) (6)
Email: iic8@cdc.gov

"To be the preeminent resource for the safety and security of biological agents and toxins."

This document is intended for the exclusive use of the recipient named above. It may contain sensitive information that is protected, privileged, or confidential, and it should not be disseminated, distributed, or copied. If you think you have received this document in error, please notify the sender immediately and destroy the original, thank you.

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: sera
Date: Monday, November 18, 2013 3:07:57 PM

Either of you two know?

M

From: Frey, Rebecca K - APHIS
Sent: Monday, November 18, 2013 2:05 PM
To: McCollum, Matthew P - APHIS
Subject: sera

Hey, Did Ryan send you any of the serum from the GonaCon cows for gonacn titers this summer? If so, did you get the results?

Thanks

Killed a cow and 3 does so far.....got meat?

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: [Rhyan, Jack C \(APHIS\)](#)
To: [Quance, Christine R \(APHIS\)](#)
Cc: [Frey, Rebecca K \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#)
Subject: FW: seropositive brucella cows in Idaho
Date: Tuesday, August 16, 2011 4:30:23 PM

Chris,

See below the info from Freeda that says we now can sample a naturally infected animal time after time and the specimen is the select agent once it is confirmed positive. We will be doing this both with some seropositive bison bulls soon and starting next spring with the contraception study. I'll give you a call and talk about the bull study. Also, who should I talk to about getting some fresh WHO media in tubes?

Jack

-----Original Message-----

From: Freeda E Isaac [<mailto:freeda.e.isaac@aphis.usda.gov>]
Sent: Thursday, May 13, 2010 11:50 AM
To: Jack C Rhyan
Subject: Fw: seropositive brucella cows in Idaho

Hi Jack,

As we had discussed over the phone several weeks ago, the Select Agent Program directors were meeting to discuss the issues related to naturally infected versus experimentally infected animals and the status of samples taken from these animals.

In our discussions, it was agreed upon that for naturally affected animals, samples taken from those animals would not be considered select agent material and required to be handled as restricted material until the sample was confirmed to have select agent material. For the issues you have raised below for the cattle you have, the samples may be handled as you have described and not subject to select agent requirements until the sample itself is confirmed positive for select agents.

For your questions regarding registration with the select agent program, you would need to have a security risk assessment completed which is a background check by FBI. That would be different than what would have been done at Plum Island previous to about 2005. I will give you a call to discuss other issues with the cattle. For information on completing the registration documents, you can call Sherylyn Roberson at 301-734-5460.

Thanks, Freeda

Freeda E. Isaac, DVM
Director
National Center for Import Export
USDA/APHIS/Veterinary Services
Phone: 301-734-8364
Fax: 301-734-6402
Email: Freeda.E.Isaac@aphis.usda.gov

----- Forwarded by Freeda E Isaac/MD/APHIS/USDA on 05/13/2010 01:35 PM -----

Freeda E Isaac/MD/APHIS/USDA
04/02/2010 12:14 PM
To
Jack C Rhyan/CO/APHIS/USDA@USDA

cc

Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject

Re: seropositive brucella cows in Idaho

Hi Jack,

Although naturally infected animals are not considered select agents themselves and not subject to the select agent regulations, once these animals are confirmed as positive for a select agent, any materials from these animals would be treated as select agent material. The infected cattle are considered the natural source of the Brucella and the materials from these animals are being intentionally collected. This is found in 9 CFR 121.3(d)(1). For example, blood, tissue specimens, urine, etc. would be subject to handling as select agent material. My understanding is these Idaho cattle have been confirmed for B. abortus by VS, therefore the materials from these cattle would need to be handled in accordance with the select agent requirements.

9 CFR 121.6(a)(1) requires that specimens are transferred to a select agent registered facility for that particular select agent within 7 calendar days. 9 CFR 121.16 describes the transfer process in which a APHIS/CDC Form 2 is completed and submitted to APHIS for approval prior to the transfer.

Let me know if you have any other questions. Freeda

Freeda E. Isaac, DVM
Director
National Center for Import Export
USDA/APHIS/Veterinary Services
Phone: 301-734-8364
Fax: 301-734-6402
Email: Freeda.E.Isaac@aphis.usda.gov

Jack C Rhyan/CO/APHIS/USDA
03/22/2010 05:44 PM
To
Freeda E Isaac/MD/APHIS/USDA@USDA

cc

Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA

Subject

seropositive brucella cows in Idaho

Freedra,

Current situation: Idaho has 4 seroconverters, at least 2 have aborted. Milk and fetus from one cow has been submitted for culture.

My proposal: I would purchase the 4 and bring to Fort Collins (with state vet's and AVIC's approval). We would then collect large amounts of blood for NVSL's serum bank. We would periodically collect urine from them; filter it to exclude bacteria and submit urine samples for GC/mass spec analysis for volatile organic compounds (VOCs) specific for brucella. We would also analyze breath for VOCs. Breath analysis and urine can be done here. Urine also might be shipped to APHIS personnel at a laboratory in PA for GC/MS analysis. I think this project would last 6 months at the end of which we would kill cows and submit specimens for culture.

Please advise me on this potential study in view of select agent requirements.

Thanks much for your help.

Jack

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: FW: seropositive brucella cows in Idaho
Date: Friday, June 14, 2013 4:00:00 PM

Actually, it did come from Freeda originally...

-----Original Message-----

From: Quance, Christine R (APHIS)
Sent: Wednesday, August 17, 2011 8:19 AM
To: Rhyan, Jack C (APHIS); Robbe Austerman, Suelee (APHIS); Henry, Lisa A (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: RE: seropositive brucella cows in Idaho

Hi Jack,

Thanks for sending this. We are registered with the CDC now instead of APHIS, but they should have the same rules.

I'll send a separate email on the media.

Chris Quance

Microbiologist-Team Leader, Mycobacteria and Brucella Section National Veterinary Services Laboratory
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.R.Quance@aphis.usda.gov

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-----Original Message-----

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, August 16, 2011 5:30 PM
To: Quance, Christine R (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: FW: seropositive brucella cows in Idaho

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Sent: Thursday, May 13, 2010 11:50 AM
To: Jack C Rhyan
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Thanks, Freeda

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McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
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Freeda E. Isaac, DVM
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Jack C Rhyan/CO/APHIS/USDA
03/22/2010 05:44 PM
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cc
Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Pauline
Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA

Subject
seropositive brucella cows in Idaho

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Current situation: Idaho has 4 seroconverters, at least 2 have aborted. Milk and fetus from one cow has been submitted for culture.
My proposal: I would purchase the 4 and bring to Fort Collins (with state vet's and AVIC's approval). We would then collect large amounts of blood for NVSL's serum bank. We would periodically collect urine from them; filter it to exclude bacteria and submit urine samples for GC/mass spec analysis for volatile organic compounds (VOCs) specific for brucella. We would also analyze breath for VOCs. Breath analysis and urine can be done here. Urine also might be shipped to APHIS personnel at a laboratory in PA for GC/MS analysis. I think this project would last 6 months at the end of which we would kill cows and submit specimens for culture.

Please advise me on this potential study in view of select agent requirements.
Thanks much for your help.
Jack

From: [Herriott, Donald E - APHIS](#)
To: [APHIS-VS WR AVIC](#)
Subject: FW: SHTP Outreach Billings MT 2013
Date: Thursday, May 30, 2013 11:45:47 AM

I know several of you are working through this process and thought this might provide some info and contacts.

Kudos to our colleague to the North, Dr. Linfield.

I'll see if "presentations" are available.

Thanks Angela for bringing this to my attn.

Donald E. Herriott
Associate Director
Western Region
2150 Centre Ave. Bldg B, MS 3E13
Ft. Collins, CO 80526
Phone: 970-494-7399
Cell: (b) (6)
Email: don.e.herriott@aphis.usda.gov

From: Carolan, Rory O - APHIS
Sent: Thursday, May 30, 2013 11:05 AM
To: Thomas, Lee A - APHIS
Cc: Astling, Joseph T - APHIS; Pelzel-McCluskey, Angela - APHIS; Porter Spalding, Barbara A - APHIS; (b) (6) [colostate.edu](#); Brewer, Becky L - APHIS; Geiser-Novotny, Sunny - APHIS; Warner, David P - APHIS; Bischoff, Barbara A - APHIS; Kasari, Ellen M - APHIS; Southall, Robert E - APHIS; Meade, Barry J - APHIS; Fox, Patricia E - APHIS; Kane, Albert J - APHIS; Carter, Michael A - APHIS; Egrie, Paul G - APHIS; Linfield, Thomas F - APHIS; Oleck, Renee S - APHIS
Subject: SHTP Outreach Billings MT 2013

Dr. Thomas,

On May 23rd and 24th 2013 Joey Astling (Slaughter Horse Compliance Specialist/Field Coordinator USDA/APHIS/VN/NCIE) and myself travelled to Billings Livestock Market, Billings MT to conduct Slaughter Horse Transportation Program training. This outreach was at the request of, and ably facilitated by, Tom Linfield, AVIC MT.

This outreach was designed to increase awareness of the recently expanded regulations that now cover horses being transported in any stage in the slaughter chain. The outreach effort's goals are summarized below:

1. Gaining knowledge of and providing outreach, education and enforcement at known horse sales, stockyards, auctions, markets, feedlots, rendering facilities and slaughter plants

2. Fostering effective working relationships with State Animal Health Offices and Investigative and Enforcement Services (IES) related to SHTP
3. Continuing SHTP outreach and education with accredited veterinarians providing CVIs and/ or export health certificates for slaughter horses
4. Reconciliation of green back tags
5. Continuing hands on enforcement of the SHTP requirements at the southern border ports with Mexico
6. Continuing training of field personal by SHTP coordinator at the request of the AVIC

We had two distinct audiences - regulatory animal health officials in the morning, and in the afternoon, members of slaughter horse industry.

The regulatory session was attended by 4 MT livestock inspectors, the MT State veterinarian, the assistant MT State veterinarian, the AVIC, a field VMO, an accredited veterinarian working out of the Billings Livestock Market, the USDA Livestock ID specialist and others.

The producer session was well attended by, among others, the horse sales professional at the market, a former MT state senator, a business consultant to the slaughter industry, the US Chairman of the International Equine Business Association (and MT state representative), commercial buyers, commercial shippers, and various private business interests and almost every employee of the Billings Livestock Market.

Joey Astling conducted the two part training. The first presentation gave the history of the SHT program, highlights of the regulation, where the program is today and where the program is going. The second presentation was a detailed examination of 9CFR part 88 with emphasis on recent changes to the rule.

Both groups were extremely receptive to the messages, attentive and full of follow up questions. The audiences were appreciative of the training, with such comments as “ ... we really learned a lot ...” and, most remarkably, from a shipper who just paid \$32,000 in fines: “ ... this has been great training, really wish it had been available a few years ago!”. Positive comments continue to come in to the AVIC’s office.



Sincerely,

Rory O. Carolan, D.V.M.
Equine Specialist
Veterinary Services
Animal Plant and Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, Maryland 20737
301.851.3558 office
(b) (6) blackberry
rory.o.carolan@aphis.usda.gov

From: [Rhyon, Jack C \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#)
Subject: FW: Some Q's on the GonaCon protocol and request for conf call
Date: Thursday, June 16, 2011 12:17:02 PM

Looks like this time may work for everyone in Riverdale. Can you all make it? It would be helpful.
Jack

From: Rhyon, Jack C (APHIS)
Sent: Thursday, June 16, 2011 10:27 AM
To: Edmundson, Jack P (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

How 'bout Tuesday the 21st at 3:30 eastern (1:30 in CO)?
Jack

From: Edmundson, Jack P (APHIS)
Sent: Tuesday, June 14, 2011 8:10 AM
To: Rhyon, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

I think Tuesday (6/21) after 1:30 (Eastern time) would be best. (Stephanie is out on 6/16 and 6/17, but will be back next week and she really should be there.) The next best day would be 6/20 (I won't be available, but Stephanie should be back and most of the others should be available then, also). – Jack E.

From: Rhyon, Jack C (APHIS)
Sent: Monday, June 13, 2011 2:37 PM
To: Edmundson, Jack P (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

Jack,
I started to answer these but decided it would be best to conf call if possible. I'm on travel; be back on Thursday. Can you all do a call Thursday or Friday?
Jack

From: Edmundson, Jack P (APHIS)
Sent: Friday, June 10, 2011 12:59 PM
To: Rhyon, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: Some Q's on the GonaCon protocol and request for conf call

Hi, Jack. We pulled the Bison Team together the other day to begin work in earnest on the GonaCon EA. The first thing we did was go through the protocol with a fine-toothed comb to be sure we understood exactly what we are planning to do. Based on some things we have seen from BFC we

suspect that they will be all over the study and watching like a hawk. As I understand it, the proposal you sent us is the final one that has been approved by NPS and a permit has been issued based on it. (In other words, APHIS shouldn't change anything in it because it would be a major paperwork hassle.) With that as background, we do have a few comments/questions about the protocol:

- How come we need a YNP permit to do work outside of the Park? And what exactly does the permit cover and not cover?

It is for the collection of bison at the trap at Stevens Creek.

- For NEPA purposes, is the lead agency APHIS or APHIS-VS? Will NPS (or NPS and APHIS-VS) officially be a cooperator in the EA? If NPS is an official cooperator, it could add additional review/approval time because NPS would have to be involved. Does NPS expect to be a NEPA Cooperator?

Concerning WS, I'll refer this question to Kathy with WS. I should visit with the Park about the EA. My guess is if they are official cooperators, it will add 3 to 10 years to the EA process.

- What is the relationship of the study to FIFRA Registration?

What is FIFRA?

- What are the roles of WS and NPS? Will they actually help in the field? Analyze info? Review/comment on things? The Park will likely work with us a little in the field. WS will also be involved a little but primarily in the lab.
- The study says it starts on June 1, 2011, presumably because we collected animals after that? From a NEPA standpoint, we would prefer to have it start in 2012 when we begin to inject animals. We have already said that NEPA did not need to be done to collect animals for research. And, if we say it has already started, then technically NEPA should already be completed. (Also, for a 7 year study, it should end in 2019, not 2017.) You're right.
- Is Cammie Johnson our statistician? Should we list her in the investigators? We haven't involved Cammie on this one.
- The 3rd Objective does not seem to have a hypothesis associated with it. Also, the only thing in the Methods/Procedures section that could relate is the paragraph talking about what is to happen if there is an abortion in the field. It is not tied together very clearly (at least not enough for us to explain it to the public, as we must do in the EA).
- In several places we talk about marking animals, but it is not real clear how. For instance on p.4 #8 we mention collars, but elsewhere we talk about ear tags and microchips. We will need to talk about which methods we use and when.
- There is some confusion in our minds about the months when things happen. For instance, on page 5 we identify a time period when bulls will be separated from cows as outside the breeding season (from Oct to July), and the abortion/calving season from Feb to Aug. These dates will allow bulls to be with cows in August, when they could be exposed to abortions/birth-related shedding.
- We were confused by the statistics section and will probably need to be walked through that so that we can understand what we are measuring and what it means.
- There is also some confusion about when we can donate to food banks, when incineration will be used, when chemicals will be used for immobilization and/or euthanasia.

There are additional small points we would want to just talk with you about to get them straight in

our minds or to ask your advice as to how to best present them in an EA. Can we organize a conference call with you to talk some of these things out? Since I am getting ready to retire, I'll be phasing out of the bison business (one of my regrets at retiring) and Stephanie Stephens will be taking my place. Since she (and Vicki) will be leading the NEPA effort, she will be getting in contact with you to set up the conference call, but we wanted you to have at least a partial list of the things we have been thinking about.

Jack E

From: [Jack C Rhyan](#)
To: [Pauline Nol](#)
Subject: Fw: study proposal and capture recipe
Date: Monday, February 28, 2011 11:28:00 AM
Attachments: [Rhyan Immunocontraception Study Plan_rlw review.doc](#)

FYI

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 02/28/2011 11:28 AM -----

Rick_Wallen@nps.gov ToJack.C.Rhyan@aphis.usda.gov
cc
02/28/2011 11:07 AM Subjectre: study proposal and capture recipe

(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

I included some thoughts and suggested edits in the attached study plan. I suggested that our Veterinary Staff in Ft. Collins be co investigators but have not received any replies from them. I will follow up with a phone call to check in with Margaret and Jenny.

We ended the field season using an Etorphine recipe with 1.3 ml high potency etorphine +0.2 ml (20 mg) xylazine (Reversed with 10 ml Naltrexone). We provide extra naltrex to prevent renarc problems in the presence of predators. If you calculate it out you can probably reverse with about 7 ml. See ZooPharm website.

RW(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria occurrence of following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission the shedding~~ of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyen et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals.

The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the

UM&R will be used-made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Commented [r1]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r2]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r3]: Why not get the culture done as soon as possible?

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in ~~reducing transmission of *B. abortus* in bison herds will be determined~~ preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector.
 - 1- Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting?
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy, whether they would have an infectious live birth, or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

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From: [Pauline Nol](#)
To: [Rebecca K Frey](#)
Subject: Fw: study proposal and capture recipe
Date: Wednesday, March 09, 2011 8:47:00 AM
Attachments: [Rhyan Immunocontraception Study Plan_rlw review.doc](#)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

----- Forwarded by Pauline Nol/CO/APHIS/USDA on 03/09/2011 08:47 AM -----

Jack C ToPauline Nol/CO/APHIS/USDA@USDA
Rhyan/CO/APHIS/USDA cc
02/28/2011 11:28 AM SubjectFw: study proposal and capture recipe

FYI

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 02/28/2011 11:28 AM -----

Rick_Wallen@nps.gov ToJack.C.Rhyan@aphis.usda.gov
cc
02/28/2011 11:07 AM Subjectre: study proposal and capture recipe

(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

I included some thoughts and suggested edits in the attached study plan. I suggested that our Veterinary Staff in Ft. Collins be co investigators but have not received any replies from them. I will follow up with a phone call to check in with Margaret and Jenny.

We ended the field season using an Etorphine recipe with 1.3 ml high potency etorphine +0.2 ml (20 mg) xylazine (Reversed with 10 ml Naltrexone). We provide extra naltrex to prevent renarc problems in the presence of predators. If you calculate it out you can probably reverse with about 7 ml. See ZooPharm website.

RW(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria occurrence of following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission the shedding~~ of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

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From: [Frey, Rebecca K - APHIS](#)
To: [Rhyon, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: update on Fort Belknap bison deaths
Date: Monday, July 06, 2015 2:49:24 PM

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Zaluski, Martin
Sent: Monday, July 6, 2015 2:19 PM
To: Mackay, Christian
Cc: TBaker@mt.gov; Tom Linfield (Thomas.F.Linfield@aphis.usda.gov); Tahnee Szymanski (tszymanski@mt.gov); Eric Liska - LIV (eliska@mt.gov); Bill Layton (blayton@mt.gov); Jennifer Ramsey (jramsey@mt.gov); Mike Volesky - FWP (MVolesky@mt.gov)
Subject: update on Fort Peck bison deaths

Christian –

Based on conversation with Mark Azure about an hour ago...

- 17 BQFS bison dead on Fort [Belknap](#) since July 4th (14 females with calves and 3 males)
- New water on Thursday July 2nd, but well water used
- Private veterinarian there today doing necropsies and testing for anthrax.
- Per Mark Azure, three anthrax test kits run. 2 are NEGATIVE, and 1 INCONCLUSIVE

I'm waiting on a call from the veterinarian. Our experience with the anthrax kits is that they are quite sensitive, but only on recently dead animals. Anthrax still a possibility, but also need to consider other potential causes such as: toxicity –plants, bad water, lead, water deprivation, MCF, or other infectious agent. Mark Azure will call me back if they see additional mortalities.

When I speak with the private veterinarian, I'll update this message accordingly. The DOL veterinarians and the FWP veterinarian (copied on this message) are in Colorado this week. We will stay tuned to the mobile phone and email.

CURRENT ISSUES:

- Fostering/feeding bison calves (born May) from dead dams to give best chance of survival
- Need to rule out anthrax and hopefully find cause of mortalities

- Need to discuss findings with private veterinarian
- Samples (anthrax and otherwise) need to be submitted to lab

Thanks,
Marty

Marty Zaluski, DVM
State Veterinarian
Montana Department of Livestock

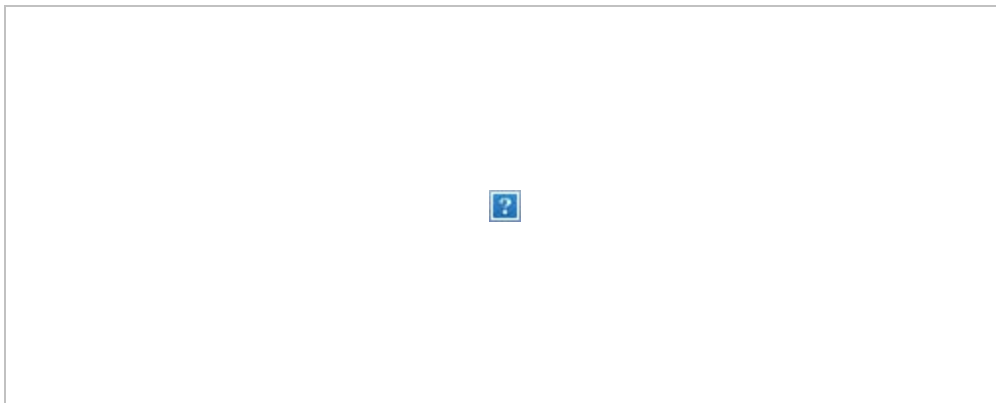
From: [Rhyan, Jack C \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: FW: Wild Buffalo Stolen from the Public
Date: Thursday, May 26, 2011 2:36:09 PM

Fun fun

From: Edmundson, Jack P (APHIS)
Sent: Thursday, May 26, 2011 11:04 AM
To: Ann M Nasr (Business Fax); Gertonson, Arnold A (APHIS); Cox, Debra C (APHIS); DARLENE BOLINGER; Donch, Debra A (APHIS); Hayes, David J (APHIS); Rhyan, Jack C (APHIS); Jack P Edmundson (Business Fax); (b) (6) @montana.com; (b) (6) @wcs.org; Kenneth Dial (Business Fax); McCluskey, Brian J (APHIS); mfrost@mt.gov; Clarke, Patrick R. (APHIS); Rivas, Jennifer M (APHIS); (b) (6); Stephens, Stephanie H (APHIS); Vicki L Wickheiser (Business Fax); Willard, Tracy A (APHIS)
Subject: FW: Wild Buffalo Stolen from the Public

From: bfc-media@wildrockies.org
Sent: Thursday, May 26, 2011 7:00 AM
To: Edmundson, Jack P (APHIS)
Subject: Wild Buffalo Stolen from the Public

[View this email in your web browser](#)



***Buffalo Field Campaign is the only group working in the field
and in the policy arena to protect America's last wild buffalo.***

[Buffalo Field Campaign](#)

Yellowstone Bison

Update from the Field

May 26, 2011



* Update from the Field

- * TAKE ACTION!
- * Spend Summer in Yellowstone with BFC!
- * BFC Wish List: Darrell Needs a New Computer
- * By the Numbers
- * Last Words

* Update from the Field



A mother and calf lead the way, running to escape those who have oppressed them. Hundreds of wild buffalo have been confined in government traps since January, and nearly 150 calves were born in captivity. BFC file photo. Click photo for larger image.

By the time you read these words, Yellowstone's Stephens Creek bison trap will be empty. Most of the groups of buffalo released from Stephens Creek fled quickly from the trap and their captors, without having to be coaxed by horsemen. On Tuesday, fifty-eight adults and about ten calves were also released from the Corwin Springs trap and thankfully, they were not loaded onto livestock trailers as we feared, and no hazing horsemen needed to tell them to flee. Patrols report that the lead cow and her calf set the pace full speed ahead and the buffalo ran over eight miles south towards Yellowstone's interior.



Young buffalo just like these were not released with their families from Corwin Springs, as Yellowstone promised they would be. They remain in captivity and have been handed over to USDA-APHIS for unknown, experimental purposes. BFC file photos by Peter Bogusko and Stephany Seay. Click photos

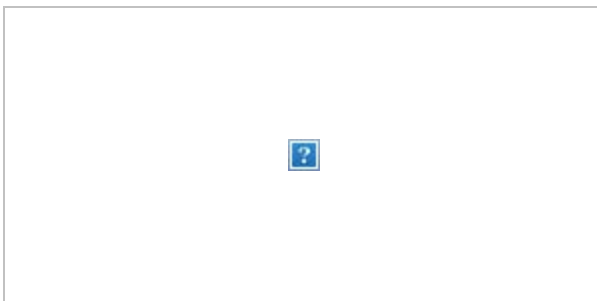
for larger images.

Unfortunately, Yellowstone didn't keep their word to release all trapped buffalo: Thirty young buffalo - yearlings and two-year olds - remain locked inside [the Corwin Springs trap](#) and will not be released. They have been surrendered to the USDA-Animal & Plant Health Inspection Service (APHIS) for unknown, experimental reasons, which BFC is working to discover. These young buffalo had to watch as their families were set free, fleeing their oppressors, while they were forced to remain behind. This is a sneaky, dishonorable, back-door move by Yellowstone, who gave no public notice, nor sought any public input, on a decision to hand over public wildlife to an agency known for maltreatment of wild buffalo. Below is an action alert to Yellowstone Superintendent Wenk, which addresses this point. We also suggest that people [contact USDA-APHIS representative Brian McCluskey](#), to ask him what APHIS intends to do with these buffalo and to let him know you oppose their possession of wild buffalo and want them set free as Yellowstone promised.



Bull buffalo run at full speed, through a neighborhood near Duck Creek, after being chased by two DOL agents on ATVs. The bulls kept running even after the agents retreated, and bolted out onto the highway. The irresponsible DOL agents gave no warning to motorists that these frightened and upset bulls could be headed in their direction. BFC was immediately on the highway putting up signs to warn traffic. BFC file photo by Stephany. Click photo to view larger image.

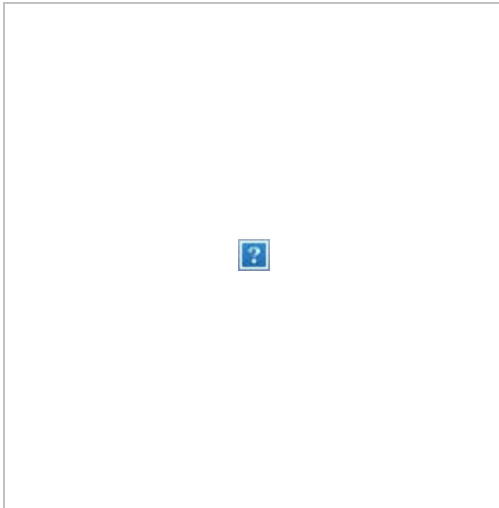
On Monday morning volunteers at camp and patrols in the field were alerted to the chop! chop! chop! of the MT Department of Livestock's (DOL) helicopter. We were in position, immediately ready for a big hazing operation, but it never came and the helicopter soon disappeared after disrupting the morning with its obnoxious presence. The DOL did end up harassing some buffalo; ten bull bison were chased off of the Koelzer property, where the Duck Creek bison trap is located. The agents aggravated the bulls into a stampede, causing them to flee the property, bolt down Duck Creek Road, and run full speed onto Highway 191 where they were lucky not to be killed by traffic.



Mama buffalo, new calves, and other family members were hazed by six government horsemen on Wednesday. Initially chased off of a private hobby ranch, the buffalo were pursued for miles by agents through Gallatin National Forest lands where there are never any cattle, and where protected grizzly bears - extremely sensitive to human commotion - are trying to make a living. BFC file photo by Stephany. Click photo for larger image.

On Wednesday, DOL and Yellowstone horsemen, along with Park Service, Forest Service, and Gallatin County law enforcement hazed a family group of buffalo, including seven newborn calves, off of the Deep Well Ranch owned by hobby rancher Pat Povah; this single

conflict area near the South Fork of the Madison River is a prime candidate for a fence, or a hired herder. Fence in the conflict and let the buffalo roam! The buffalo were pursued by six horsemen for over nine miles through the thick timber of cattle-free Gallatin National Forest lands into habitat where grizzly bears are currently active. Another group of buffalo, including three new calves, were also caught up in the haze. Agent riders continued hazing the buffalo north on Highway 191, across the Madison River, then into Yellowstone for nearly two more miles. Government bison hazing operations that harm buffalo and adversely impact protected grizzly bears are currently being legally challenged by the Alliance for the Wild Rockies. [Visit BFC's home page to learn about this law suit.](#)



Some new calves have had a little more time than usual to develop and grow strong before the government's big hazing operations begin. As calving season is still in its peak, other baby buffalo as well as nursing or pregnant mothers will not be so lucky when the Montana Department of Livestock's helicopter descends upon this fragile, wildlife-rich ecosystem. BFC file photo by Stephany. Click photo for larger image.

For this week, Yellowstone National Park has refused to allow the DOL to haze buffalo deep into Yellowstone due to lingering snowpack and flooding conditions that have slowed green-up on the buffalo's summer range. We do, however, anticipate big hazing operations to begin next week. Grizzly bears remain very active in the area, while some buffalo are still recovering from extreme winter conditions. Many buffalo have yet to give birth and calves are still being born every day. Hazing operations are disruptive to all area wildlife but they take a particularly heavy toll on on pregnant buffalo and newborn calves, even causing injury and death, as sadly demonstrated in [this BFC video](#).

Please see below to take action for America's last wild buffalo. Your continued actions over the years have made a huge difference, and this year helped prevent the slaughter of nearly 800 wild buffalo, helped gain some new habitat access, helped force decision-makers to rethink the status quo, and helped draw attention to the senseless acts of violence against these last wild buffalo. Change is slow to come, but it is on the way. Thank you!

ROAM FREE!
~Stephany

*** TAKE ACTION!**



Bull buffalo challenge one another for the opportunity to court females; wild bison advocates must challenge government agencies and livestock interests for the right of all wild buffalo to roam free. BFC file photo by Darrell Geist. Click photo to view larger image.

1. Contact Gallatin National Forest Supervisor Mary Erickson and demand that she enforce the Forest Service's promise to the public that "Currently, hazing operations would cease if there was evidence of grizzlies being active in the area" (IBMP EIS page 565). There are grizzly bears all over the Gallatin National Forest west of Yellowstone National Park right now, so she should not allow the DOL to engage in hazing operations there. Please insist that she allow buffalo year-round habitat on the Gallatin National Forest. **[TAKE ACTION!](#)**

2. Contact Yellowstone Superintendent Dan Wenk. Thank him for releasing most buffalo, and demand to know why the Park surrendered 30 young wild buffalo to APHIS for experimental purposes. Remind him of the Park's responsibility to protect grizzly bears from impacts of hazing, and tell him to stay the course and not allow the DOL to haze buffalo into Yellowstone. Please also ask him to facilitate a National Park Service response to the letter from Congress. As always, continue to encourage him to protect America's last wild buffalo population, not cater to Montana's livestock interests. In addition to sending an electronic message you can call Superintendent Wenk at 307-344-2003. **[TAKE ACTION!](#)**

3. Contact Your Members of Congress and urge them to bring an end to the Interagency Bison Management Plan and re-direct federal funding towards habitat-based solutions for wild buffalo. If your Members of Congress are unfamiliar with this issue, urge them to contact Buffalo Field Campaign. **[TAKE ACTION!](#)**

4. Write Letters to the Editor in support of protecting America's last wild buffalo. **[TAKE ACTION!](#)**

Thank You! Spread the word to save these herds! Please share these action links on FaceBook, blogs, etc.

*** Spend Summer in Yellowstone with BFC!**

BFC's summer tabling crew is forming, and we could use a few more volunteers. Come to Yellowstone country and help us talk with millions of visitors to Yellowstone and Grand Teton National Parks. BFC provides food, lodging, camping in and near the Park, gear, and transportation to and from our table in the Park. We ask for at least a three week commitment in order to accommodate training and orientation. This is a great opportunity to get involved and advocate for America's last wild buffalo! Please [contact Tony](#) for more information.

*** BFC Wish List: Darrell Needs a New Computer!!**

BFC's stellar and hard-working Habitat Coordinator, Darrell Geist, is in need of a new computer. Darrell's current computer has been a faithful work horse for years, helping Darrell craft important briefing papers, develop extensive public comments, engage in solidarity work with Tribal interests, draft legal strategies, conduct vast amounts of research, disseminate critical information to colleagues and decision-makers, and has been a critical tool in keeping wild buffalo and their need to access habitat in the face of everyone. Having given all its able to give, Darrell's computer is ready to retire.

Please help BFC's Habitat Coordinator acquire a new computer! Darrell's dream machine can be [found here](#).

*** By the Numbers**

AMERICAN BUFFALO ELIMINATED from the last wild population in the U.S. The last wild population is currently estimated at fewer than 3,600 individual buffalo.

2010-2011 Total Buffalo Killed: 227

2010-2011 Government Capture: 770

2010-2011 Government Slaughter: 0

2010-2011 Held for Government Experiment: 30

2010-2011 Died In Government Trap: 3

2010-2011 Miscarriage in Government Trap: 1

2010-2011 State & Treaty Hunts: 211

2010-2011 Quarantine: 0

2010-2011 Shot by Agents: 3

2010-2011: Killed by Angry Residents: 2

2010-2011 Highway Mortality: 9

2009-2010 Total: 7

2008-2009 Total: 22

2007-2008 Total: 1,631

* Total Since 2000: 3,969*

*includes lethal government action, trap-related fatalities, quarantine, hunts, highway mortality

*** Last Words ~ J.R.R. Tolkein**

*"All things worthy that are in peril,
as the world now stands,
those are my care."*

~ J.R.R. Tolkein

Do you have submissions for Last Words? Send them to [Stephany](#). Thank you for all the poems, songs, quotes, and stories you have been sending; you'll see them here!

--

Media & Outreach
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
bfc-media@wildrockies.org
<http://www.buffalofieldcampaign.org>

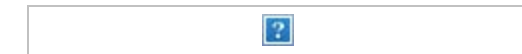
***BFC is the only group working in the field every day
in defense of the last wild buffalo population in the U.S.***

[KEEP BFC ON THE FRONTLINES](#)

[Join Buffalo Field Campaign](#) -- It's Free!

[Tell-a-Friend!](#)

[Take Action!](#)



ROAM FREE!



[Click here to unsubscribe.](#)



From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#)
Subject: FW: QA number
Date: Friday, December 30, 2011 10:24:00 AM

Never mind, I found the QA number for this. 1858

Thanks!

From: Nol, Pauline - APHIS
Sent: Friday, December 30, 2011 10:11 AM
To: Greiner, Laura B - APHIS
Subject: QA number



Hi Laura,

I either need a new QA# for the study: **Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

or I need to be reminded of it☺

Thanks and happy New Year!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6138

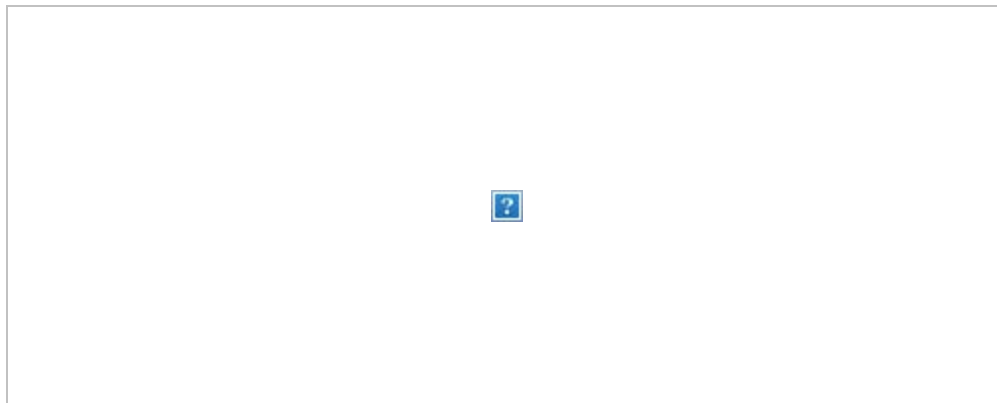
From: [Rhyan, Jack C \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: FW: Wild Buffalo Stolen from the Public
Date: Thursday, May 26, 2011 2:36:09 PM

Fun fun

From: Edmundson, Jack P (APHIS)
Sent: Thursday, May 26, 2011 11:04 AM
To: Ann M Nasr (Business Fax); Gertonson, Arnold A (APHIS); Cox, Debra C (APHIS); DARLENE BOLINGER; Donch, Debra A (APHIS); Hayes, David J (APHIS); Rhyan, Jack C (APHIS); Jack P Edmundson (Business Fax); (b) (6) @montana.com; (b) (6) @wcs.org; Kenneth Dial (Business Fax); McCluskey, Brian J (APHIS); mfrost@mt.gov; Clarke, Patrick R. (APHIS); Rivas, Jennifer M (APHIS); (b) (6); Stephens, Stephanie H (APHIS); Vicki L Wickheiser (Business Fax); Willard, Tracy A (APHIS)
Subject: FW: Wild Buffalo Stolen from the Public

From: bfc-media@wildrockies.org
Sent: Thursday, May 26, 2011 7:00 AM
To: Edmundson, Jack P (APHIS)
Subject: Wild Buffalo Stolen from the Public

[View this email in your web browser](#)



***Buffalo Field Campaign is the only group working in the field
and in the policy arena to protect America's last wild buffalo.***

[Buffalo Field Campaign](#)

Yellowstone Bison

Update from the Field

May 26, 2011



* Update from the Field

- * TAKE ACTION!
- * Spend Summer in Yellowstone with BFC!
- * BFC Wish List: Darrell Needs a New Computer
- * By the Numbers
- * Last Words

* Update from the Field



A mother and calf lead the way, running to escape those who have oppressed them. Hundreds of wild buffalo have been confined in government traps since January, and nearly 150 calves were born in captivity. BFC file photo. Click photo for larger image.

By the time you read these words, Yellowstone's Stephens Creek bison trap will be empty. Most of the groups of buffalo released from Stephens Creek fled quickly from the trap and their captors, without having to be coaxed by horsemen. On Tuesday, fifty-eight adults and about ten calves were also released from the Corwin Springs trap and thankfully, they were not loaded onto livestock trailers as we feared, and no hazing horsemen needed to tell them to flee. Patrols report that the lead cow and her calf set the pace full speed ahead and the buffalo ran over eight miles south towards Yellowstone's interior.



Young buffalo just like these were not released with their families from Corwin Springs, as Yellowstone promised they would be. They remain in captivity and have been handed over to USDA-APHIS for unknown, experimental purposes. BFC file photos by Peter Bogusko and Stephany Seay. Click photos

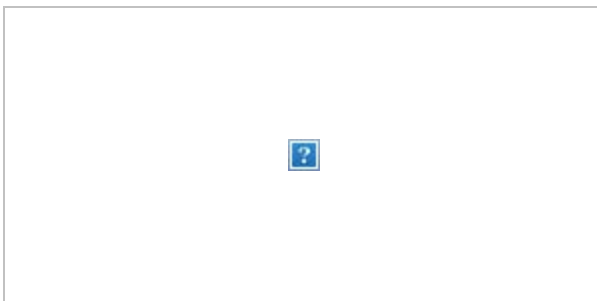
for larger images.

Unfortunately, Yellowstone didn't keep their word to release all trapped buffalo: Thirty young buffalo - yearlings and two-year olds - remain locked inside [the Corwin Springs trap](#) and will not be released. They have been surrendered to the USDA-Animal & Plant Health Inspection Service (APHIS) for unknown, experimental reasons, which BFC is working to discover. These young buffalo had to watch as their families were set free, fleeing their oppressors, while they were forced to remain behind. This is a sneaky, dishonorable, back-door move by Yellowstone, who gave no public notice, nor sought any public input, on a decision to hand over public wildlife to an agency known for maltreatment of wild buffalo. Below is an action alert to Yellowstone Superintendent Wenk, which addresses this point. We also suggest that people [contact USDA-APHIS representative Brian McCluskey](#), to ask him what APHIS intends to do with these buffalo and to let him know you oppose their possession of wild buffalo and want them set free as Yellowstone promised.



Bull buffalo run at full speed, through a neighborhood near Duck Creek, after being chased by two DOL agents on ATVs. The bulls kept running even after the agents retreated, and bolted out onto the highway. The irresponsible DOL agents gave no warning to motorists that these frightened and upset bulls could be headed in their direction. BFC was immediately on the highway putting up signs to warn traffic. BFC file photo by Stephany. Click photo to view larger image.

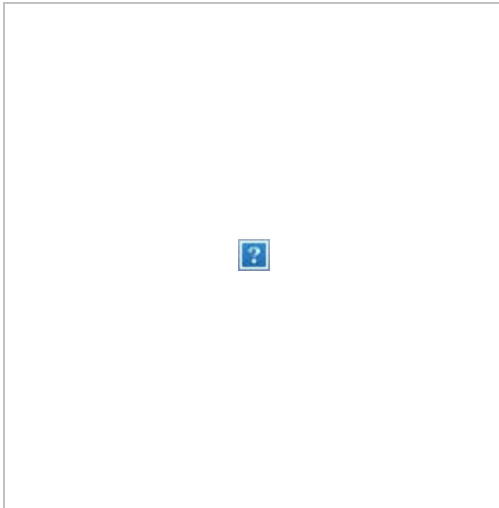
On Monday morning volunteers at camp and patrols in the field were alerted to the chop! chop! chop! of the MT Department of Livestock's (DOL) helicopter. We were in position, immediately ready for a big hazing operation, but it never came and the helicopter soon disappeared after disrupting the morning with its obnoxious presence. The DOL did end up harassing some buffalo; ten bull bison were chased off of the Koelzer property, where the Duck Creek bison trap is located. The agents aggravated the bulls into a stampede, causing them to flee the property, bolt down Duck Creek Road, and run full speed onto Highway 191 where they were lucky not to be killed by traffic.



Mama buffalo, new calves, and other family members were hazed by six government horsemen on Wednesday. Initially chased off of a private hobby ranch, the buffalo were pursued for miles by agents through Gallatin National Forest lands where there are never any cattle, and where protected grizzly bears - extremely sensitive to human commotion - are trying to make a living. BFC file photo by Stephany. Click photo for larger image.

On Wednesday, DOL and Yellowstone horsemen, along with Park Service, Forest Service, and Gallatin County law enforcement hazed a family group of buffalo, including seven newborn calves, off of the Deep Well Ranch owned by hobby rancher Pat Povah; this single

conflict area near the South Fork of the Madison River is a prime candidate for a fence, or a hired herder. Fence in the conflict and let the buffalo roam! The buffalo were pursued by six horsemen for over nine miles through the thick timber of cattle-free Gallatin National Forest lands into habitat where grizzly bears are currently active. Another group of buffalo, including three new calves, were also caught up in the haze. Agent riders continued hazing the buffalo north on Highway 191, across the Madison River, then into Yellowstone for nearly two more miles. Government bison hazing operations that harm buffalo and adversely impact protected grizzly bears are currently being legally challenged by the Alliance for the Wild Rockies. [Visit BFC's home page to learn about this law suit.](#)



Some new calves have had a little more time than usual to develop and grow strong before the government's big hazing operations begin. As calving season is still in its peak, other baby buffalo as well as nursing or pregnant mothers will not be so lucky when the Montana Department of Livestock's helicopter descends upon this fragile, wildlife-rich ecosystem. BFC file photo by Stephany. Click photo for larger image.

For this week, Yellowstone National Park has refused to allow the DOL to haze buffalo deep into Yellowstone due to lingering snowpack and flooding conditions that have slowed green-up on the buffalo's summer range. We do, however, anticipate big hazing operations to begin next week. Grizzly bears remain very active in the area, while some buffalo are still recovering from extreme winter conditions. Many buffalo have yet to give birth and calves are still being born every day. Hazing operations are disruptive to all area wildlife but they take a particularly heavy toll on on pregnant buffalo and newborn calves, even causing injury and death, as sadly demonstrated in [this BFC video](#).

Please see below to take action for America's last wild buffalo. Your continued actions over the years have made a huge difference, and this year helped prevent the slaughter of nearly 800 wild buffalo, helped gain some new habitat access, helped force decision-makers to rethink the status quo, and helped draw attention to the senseless acts of violence against these last wild buffalo. Change is slow to come, but it is on the way. Thank you!

ROAM FREE!
~Stephany

*** TAKE ACTION!**



Bull buffalo challenge one another for the opportunity to court females; wild bison advocates must challenge government agencies and livestock interests for the right of all wild buffalo to roam free. BFC file photo by Darrell Geist. Click photo to view larger image.

1. Contact Gallatin National Forest Supervisor Mary Erickson and demand that she enforce the Forest Service's promise to the public that "Currently, hazing operations would cease if there was evidence of grizzlies being active in the area" (IBMP EIS page 565). There are grizzly bears all over the Gallatin National Forest west of Yellowstone National Park right now, so she should not allow the DOL to engage in hazing operations there. Please insist that she allow buffalo year-round habitat on the Gallatin National Forest. **[TAKE ACTION!](#)**

2. Contact Yellowstone Superintendent Dan Wenk. Thank him for releasing most buffalo, and demand to know why the Park surrendered 30 young wild buffalo to APHIS for experimental purposes. Remind him of the Park's responsibility to protect grizzly bears from impacts of hazing, and tell him to stay the course and not allow the DOL to haze buffalo into Yellowstone. Please also ask him to facilitate a National Park Service response to the letter from Congress. As always, continue to encourage him to protect America's last wild buffalo population, not cater to Montana's livestock interests. In addition to sending an electronic message you can call Superintendent Wenk at 307-344-2003. **[TAKE ACTION!](#)**

3. Contact Your Members of Congress and urge them to bring an end to the Interagency Bison Management Plan and re-direct federal funding towards habitat-based solutions for wild buffalo. If your Members of Congress are unfamiliar with this issue, urge them to contact Buffalo Field Campaign. **[TAKE ACTION!](#)**

4. Write Letters to the Editor in support of protecting America's last wild buffalo. **[TAKE ACTION!](#)**

Thank You! Spread the word to save these herds! Please share these action links on FaceBook, blogs, etc.

*** Spend Summer in Yellowstone with BFC!**

BFC's summer tabling crew is forming, and we could use a few more volunteers. Come to Yellowstone country and help us talk with millions of visitors to Yellowstone and Grand Teton National Parks. BFC provides food, lodging, camping in and near the Park, gear, and transportation to and from our table in the Park. We ask for at least a three week commitment in order to accommodate training and orientation. This is a great opportunity to get involved and advocate for America's last wild buffalo! Please [contact Tony](#) for more information.

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P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
bfc-media@wildrockies.org
<http://www.buffalofieldcampaign.org>

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[Tell-a-Friend!](#)

[Take Action!](#)



ROAM FREE!



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From: Frey, Rebecca K - APHIS
Sent: Tuesday, November 28, 2017 8:03 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Wehtje, Morgan E - APHIS
Subject: Fwd: bison
Attachments: Critical Brucellosis research in MT and CO being.docx; ATT00001.htm

Sent from my iPhone

Begin forwarded

Article from this week's Western Ag Reporter

From: Brent Thompson [mailto:(b) (6) gmail.com]
Sent: Tuesday, November 28, 2017 7:42 AM
To: Thompson, Brent D - APHIS <Brent.D.Thompson@aphis.usda.gov>
Subject: bison

From: WILLIAM.SCHMIDT@NYS.GOV
 To: J.P.McMILLIN@NYS.GOV
 Re: [WILLIAM.SCHMIDT@NYS.GOV](#) - [J.P.McMILLIN@NYS.GOV](#) - [J.P.McMILLIN@NYS.GOV](#) - [J.P.McMILLIN@NYS.GOV](#)
 Subject: RE: J.P.McMILLIN@NYS.GOV
 Date: Wed, 20 Jun 2012 17:30:00

From: [Clarke, Patrick R. - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Fwd: Additional research bison
Date: Thursday, February 27, 2014 10:33:43 PM

FYI

Sent from my iPhone

Begin forwarded message:

From: "Hallac, Dave" <david_hallac@nps.gov>
Date: February 27, 2014 at 7:10:40 PM MST
To: "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>
Cc: PJ White <pj_white@nps.gov>, Rick Wallen <rick_wallen@nps.gov>
Subject: Re: Additional research bison

Hi Ryan

We would be happy to consider this request. I'll have PJ or Rick contact you to discuss further. Thanks, Dave

David E. Hallac
Chief, Yellowstone Center for Resources
Yellowstone National Park, WY 82190
307-344-2203

<http://www.nps.gov/yell/naturescience/index.htm>

On Mon, Feb 24, 2014 at 5:25 PM, Clarke, Patrick R. - APHIS
<Patrick.R.Clarke@aphis.usda.gov> wrote:

Dave,

Please find attached a supplement to our GonaCon MOU. Our fellow APHIS scientists in Ft Collins would like ~ 15 bison for a study they are conducting investigating *B. abortus* in relationship to embryo transfer.....and with the Stephens Creek facility in full operation, they see an opportunity to get the animals they need.

We realize this is last minute and we don't like to operate in a rush any more than you do, but we would be grateful if you could please consider this supplemental MOU. Please call me on my cell (b) (6) if I can provide any further information.

Thanks, Dave.

Ryan

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA-APHIS-VS-WR

406-388-5162

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From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: Fwd: Bison to CO - only VS 1-27 required by CO
Date: Wednesday, June 17, 2015 7:03:27 AM

Could one of you provide any more information for Tom? Thanks to AI, nobody in MT for a health.

Sent from my iPhone

Begin forwarded message:

From: "Linfield, Thomas F - APHIS" <Thomas.F.Linfield@aphis.usda.gov>
Date: June 16, 2015 at 9:20:23 PM MDT
To: "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>
Cc: "Bailey, Glen R - APHIS" <Glen.R.Bailey@aphis.usda.gov>, "Thompson, Brent D - APHIS" <Brent.D.Thompson@aphis.usda.gov>, "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>
Subject: Re: Bison to CO - only VS 1-27 required by CO

The question arose today, primarily because Glen is not deputized in m MT / does not have any MT DOL issued CVI's. We tried calling Dr Roehr to confirm - he was out of town, however Dr Heckendorf acting in his absence authorized VS 1-27 only, along with obtaining a CO permit. He actually had concerns regarding issuing a CVI with the "statements of health/ lack of exposure to infectious, contagious diseases on bison known to be infected or exposed to Brucella. ...

Did Dr Roehr provide any logical rationale for the CVI in addition to the VS 1-27??? Tom

Thomas F.T. Linfield, DVM

Assistant District Director
District 5 Field Office for MT
USDA-APHIS-Veterinary Services

[208 N. Montana Ave](#); Suite 101

[Helena, MT 59601](#)

[\(406\) 449-2220](tel:(406)449-2220)

[\(b\) \(6\)](tel:(b)(6)) (cell)

[\(406\) 449-5439](tel:(406)449-5439) FAX

Thomas.F.Linfield@aphis.usda.gov

On Jun 16, 2015, at 4:34 PM, Frey, Rebecca K - APHIS
<Rebecca.K.Frey@aphis.usda.gov> wrote:

Thanks Tom,
However, Jack/ Pauline have been handling this with Dr. Roehr for several years and he always asks for a CVI. They have already informed him of the import.

Sent from my iPhone

On Jun 16, 2015, at 3:55 PM, Linfield, Thomas F - APHIS
<Thomas.F.Linfield@aphis.usda.gov> wrote:

Glen, Becky, Ryan, Brent:

Regarding sending bison from Corwin Springs to Ft. Collins, and the potential need for both CVI and VS Form 1-27: Glen stopped by the office today – we contacted Dr. Carl Heckendorf with the Colorado Department of Agriculture. Since the bison movement will be on a VS 1-27, he indicated a CVI was not necessary. He did however, request obtaining a Colorado Import number, and forwarding a scan of the VS 1-27.

Colorado Permit number: **303-869-9131**

He suggested sending a scan of the VS 1-27 to:

<!--[if !supportLists]-->1) <!--[endif]-->Keith Roehr:

Keith.Roehr@state.co.us

<!--[if !supportLists]-->2) <!--[endif]-->Carl Heckendorf:

carl.heckendorf@state.co.us

<!--[if !supportLists]-->3) <!--[endif]-->Sunny Geiser-

Novotny: Sunny.Geiser-Novotny@aphis.usda.gov

Thomas F.T. Linfield, DVM

Assistant District Director

District 5 Field Office for MT

USDA-APHIS-Veterinary Services

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Helena, MT 59601

(406) 449-2220

(b) (6) (cell)

(406) 449-5439 FAX

Thomas.F.Linfield@aphis.usda.gov

From: [Roehr - CDA, Keith](#)
To: [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [Patricia Menchaca - CDA](#); [Cristy Dice](#)
Subject: Fwd: Health Cert./bison
Date: Thursday, January 09, 2014 10:42:00 AM

Pauline and Rebecca,

We are good to go. The permit number is 09KX01-01

Keith A. Roehr DVM
Colorado State Veterinarian
[303-239-4166](tel:303-239-4166)

Please note that my email address has changed to : keith.roehr@state.co.us

----- Forwarded message -----

From: **Frey, Rebecca K - APHIS** <Rebecca.K.Frey@aphis.usda.gov>
Date: Thu, Jan 9, 2014 at 6:48 AM
Subject: Health Cert./bison
To: "keith.roehr@state.co.us" <keith.roehr@state.co.us>

Attached is the health certificate for bison from Montana, re: Dr. Nol. 1-27 to follow this morning.

Thank you

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

[406-333-4425](tel:406-333-4425)

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recipients. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the violator to civil or criminal penalties. If you believe you have received this message in error, please notify the sender and delete the email immediately.

We are good to go

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: Fwd: NVSL Report - Accession#14-010351,Purpose:GEN_DIAG,Exam Req:BRUC sent to nvsl.vs.mt@aphis.usda.gov
Date: Tuesday, August 12, 2014 3:02:34 PM
Attachments: [14-010351_DBL-BRUC_ALT_07-28-2014-11-53-02-AM.pdf](#)
[ATT00001.htm](#)
[YNP_Bison_NVSL_Accession_14-010351_Feb-Mar_2014.pdf](#)
[ATT00002.htm](#)

Sent from my iPhone

Begin forwarded message:

From: "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>
Date: July 31, 2014 at 10:44:33 MDT
To: "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov>
Subject: Fwd: NVSL Report - Accession#14-010351,Purpose:GEN_DIAG,Exam Req:BRUC sent to nvsl.vs.mt@aphis.usda.gov

Becky
USDA APHIS VS
Sent from my iPhone

Begin forwarded message:

From: "Linfield, Thomas F - APHIS" <Thomas.F.Linfield@aphis.usda.gov>
Date: July 31, 2014 at 9:18:20 AM MDT
To: "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>, "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>, "Thompson, Brent D - APHIS" <Brent.D.Thompson@aphis.usda.gov>, "Johnson, Kammy R - APHIS" <Kammy.R.Johnson@aphis.usda.gov>, "mzaluski@mt.gov" <mzaluski@mt.gov>
Subject: FW: NVSL Report - Accession#14-010351,Purpose:GEN_DIAG,Exam Req:BRUC sent to nvsl.vs.mt@aphis.usda.gov

[FYI...NVSL Results - YNP Bison from Feb/Mar 2014](#)

Thomas F.T. Linfield, DVM
Assistant District Director-Montana
USDA-APHIS-Veterinary Services

District 5 (MT, ID, WY, ND, SD, NE, KS)
208 N. Montana Ave; Suite 101
Helena, MT 59601
(406) 449-2220
(406) 449-5439 FAX

Thomas.F.Linfield@aphis.usda.gov

From: Linfield, Thomas F - APHIS

Sent: Thursday, July 31, 2014 8:54 AM

To: 'John_Treanor@nps.gov'

Subject: FW: NVSL Report - Accession#14-010351,Purpose:GEN_DIAG,Exam
Req:BRUC sent to nvsl.vs.mt@aphis.usda.gov

John:

Not sure if you received these results yet from NVSL. Attached is
the Final Report and Accession Form.

Thanks...Tom

Thomas F.T. Linfield, DVM

Assistant District Director-Montana

USDA-APHIS-Veterinary Services

District 5 (MT, ID, WY, ND, SD, NE, KS)

208 N. Montana Ave; Suite 101

Helena, MT 59601

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(406) 449-5439 FAX

Thomas.F.Linfield@aphis.usda.gov

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS

Sent: Monday, July 28, 2014 11:01 AM

To: APHIS-NVSL VS MT

Subject: NVSL Report - Accession#14-
010351,Purpose:GEN_DIAG,Exam Req:BRUC sent to
nvsl.vs.mt@aphis.usda.gov

Submitter Name: John Treanor

Submitter Company: Yellowstone National Park

Yellowstone Center for Resources

Referral Number:

FAD Number:

Accession: 14-010351

Date Received: 04/01/2014 10:59:43 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: WY

Owner State:

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Accession Number: 14-010351

Animal Location

Park County MT, US

Date Collected:

Date Received: 04/01/2014

Submitter - 4932

John Treanor

Date Completed: 07/28/2014

Collected By: John Treanor

Yellowstone National Park

Purpose: General Diagnostic

Yellowstone Center for Resources

PO Box 168

Yellowstone National Park, WY 82190

Referral Number:

This is not a billable case.

FAX #: 307-344-2014

Phone #: 307-344-2505

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 022714-01 **Animal ID:** 6642 **Brucella Case Number:** B14-0213 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-02 **Animal ID:** 6636 **Brucella Case Number:** B14-0214 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Brucella Identification Result

Isolate Determined

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-03 **Animal ID:** 6710 **Brucella Case Number:** B14-0215 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-04 **Animal ID:** 6700 **Brucella Case Number:** B14-0216 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-05 **Animal ID:** 6691 **Brucella Case Number:** B14-0217 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-06 **Animal ID:** 6718 **Brucella Case Number:** B14-0218 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-07 **Animal ID:** 6671 **Brucella Case Number:** B14-0219 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-08 **Animal ID:** 6684 **Brucella Case Number:** B14-0220 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-09 **Animal ID:** 6732 **Brucella Case Number:** B14-0221 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-10 **Animal ID:** 6715 **Brucella Case Number:** B14-0222 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-11 **Animal ID:** 6740 **Brucella Case Number:** B14-0223 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-12 **Animal ID:** 6730 **Brucella Case Number:** B14-0224 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-13 **Animal ID:** 6675 **Brucella Case Number:** B14-0225 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-14 **Animal ID:** 6699 **Brucella Case Number:** B14-0226 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-15 **Animal ID:** 6639 **Brucella Case Number:** B14-0227 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-16 **Animal ID:** 6701 **Brucella Case Number:** B14-0228 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-17 **Animal ID:** 6719 **Brucella Case Number:** B14-0229 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-18 **Animal ID:** 6706 **Brucella Case Number:** B14-0230 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-19 **Animal ID:** 6688 **Brucella Case Number:** B14-0231 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Suspect Isolated

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-20 **Animal ID:** 6746 **Brucella Case Number:** B14-0232 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-21 **Animal ID:** 6689 **Brucella Case Number:** B14-0233 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-22 **Animal ID:** 6681 **Brucella Case Number:** B14-0234 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-23 **Animal ID:** 6692 **Brucella Case Number:** B14-0235 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 022714-24 **Animal ID:** 6736 **Brucella Case Number:** B14-0236 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: Y031 **Animal ID:** 6614 **Brucella Case Number:** B14-0237 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-01 **Animal ID:** 6793 **Brucella Case Number:** B14-0238 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-02 **Animal ID:** 6768 **Brucella Case Number:** B14-0239 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Brucella Identification Result

Isolate Determined

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-03 **Animal ID:** 6766 **Brucella Case Number:** B14-0240 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Brucella Identification Result

Isolate Determined

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Sample: 030514-04 **Animal ID:** 6797 **Brucella Case Number:** B14-0241 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-05 **Animal ID:** 6779 **Brucella Case Number:** B14-0242 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-06 **Animal ID:** 6762 **Brucella Case Number:** B14-0243 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-07 **Animal ID:** 6754 **Brucella Case Number:** B14-0244 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

Suspect Isolated

Sample: 030514-08 **Animal ID:** 6794 **Brucella Case Number:** B14-0245 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-09 **Animal ID:** 6764 **Brucella Case Number:** B14-0246 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-10 **Animal ID:** 6757 **Brucella Case Number:** B14-0247 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tissue / Mammary Gland

Brucella Isolation Result

Suspect Isolated

Sample: 030514-11 **Animal ID:** 6760 **Brucella Case Number:** B14-0248 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-12 **Animal ID:** 6805 **Brucella Case Number:** B14-0249 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-13 **Animal ID:** 6778 **Brucella Case Number:** B14-0250 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-14 **Animal ID:** 6792 **Brucella Case Number:** B14-0251 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Sample: 030514-15 **Animal ID:** 6775 **Brucella Case Number:** B14-0252 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Lymph Node / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Internal Iliac

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

[Help Us Help You](#)

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
P.O. BOX 844, 1920 DAYTON AVENUE, AMES, IA 50010
(515) 337-7514

SPECIMEN SUBMISSION

PAGE
OF

INSTRUCTIONS: Use a separate form for each species and each owner/broker. See "Instructions for Completing VS Form 10-4" for definitions.

1. SUBMITTER NAME (including Business Name)

John Treanor

2. NVSL SUBMITTER ID

3. NAME OF OWNER

☒ Check if wildlife (no owner)

EMAIL ADDRESS

john_treanor@nps.gov

OWNER CITY

STATE/COUNTRY

PHONE NO. 307-344-2505

FAX NO.

MAILING ADDRESS (Street, City, State, ZIP Code)

PO Box 168, Yellowstone Center for Resources
Yellowstone National Park, WY 82190

PREMISES ID

COUNTY

Park

STATE/COUNTRY

MT/USA

☐ USER FEE ACCOUNT NO.

☐ CHECK/MONEY ORDER
(Enclosed, payable to USDA in US dollars)

☐ CREDIT CARD
Number:
Exp. Date:

6. HERD/FLOCK SIZE

9. EXAMINATIONS REQUESTED

Brucella abortus culture

7. NO. IN HERD/FLOCK AFFECTED

8. NO. IN HERD/FLOCK DEAD

10. COLLECTED BY
John Treanor

11. DATE COLLECTED
2/27/2014 & 3/5/2014

12. AUTHORIZED BY
John Treanor

14. COUNTRY OF ORIGIN/DESTINATION

15. REFERRAL NUMBER

13. PURPOSE OF SUBMISSION (See instructions for definitions)

☐ Interstate Movement

☐ Import

☐ TB

☐ Reagent Evaluation

☐ Export

☐ FAD/EP Diagnostic

☒ General Diagnostic

☐ NVSL Intralab

☐ Pre-Import

☐ Surveillance

☐ Developmental Research

16. PRESERVATION

☐ None ☐ Ice Pack ☒ Dry Ice ☐ Formalin ☐ Borax ☐ Alcohol ☐ Other (Specify)

17. SPECIMENS SUBMITTED ("X" applicable item(s))

☐ Blood

☐ Feces

☐ Parasite

☐ Serum

☒ Tissue (specify)

☐ Whole Animal

☐ Other (specify)

☐ Culture

☐ Feed

☐ Plant

☐ Soil

☐ Urine

☐ Fetus

lymph nodes and
mammary gland

☐ Extract

☐ Milk

☐ Semen

☐ Swab (specify)

☐ Water

☐ DNA/RNA

19. SPECIES OR SOURCE ("X" ONLY one)

☐ Cattle

☐ Goat

☐ Chicken

☒ Bison

☐ Fish

☐ Other (specify)

☐ Swine

☐ Horse

☐ Turkey

☐ Deer (specify)

☐ Environment

☐ Sheep

☐ Donkey

☐ Other bird (specify)

☐ Elk

☐ Reagent

18. TOTAL NUMBER OF SPECIMENS SUBMITTED

160 tissues for culture

20. NUMBER OF ANIMALS SAMPLED

40

Sample ID

Animal ID

Breed

Age

Sex

Sample ID

Animal ID

Breed

Age

Sex

22. ADDITIONAL DATA (History, clinical signs, post mortem findings, remarks, tentative diagnosis, special instructions. Use additional sheets if necessary).

All animals previously tested positive on FPA and card

SAMPLE NOT VERIFIED

23. SIGNATURE OF SUBMITTER AND DATE

X

NVSL USE ONLY

CONDITION

PRIORITY

DISTRIBUTION

RECEIVED BY

VS FORM 10-4
AUG 2009

14-010351
DBL-BRUC

04/01/2014
20615

Bison Tissue Samples for *Brucella abortus* Culture

Submitted by John Treanor, Yellowstone National Park, 307-344-2505

Slaughter.ID	Backtag	Date	Species	Sex	Age.Est
022714-01	6642	2/27/2014	Bison	F	adult
022714-02	6636	2/27/2014	Bison	F	adult
022714-03	6710	2/27/2014	Bison	F	NA
022714-04	6700	2/27/2014	Bison	F	adult
022714-05	6691	2/27/2014	Bison	F	adult
022714-06	6718	2/27/2014	Bison	F	adult
022714-07	6671	2/27/2014	Bison	F	adult
022714-08	6684	2/27/2014	Bison	F	adult
022714-09	6732	2/27/2014	Bison	F	adult
022714-10	6715	2/27/2014	Bison	F	adult
022714-11	6740	2/27/2014	Bison	F	adult
022714-12	6730	2/27/2014	Bison	F	adult
022714-13	6675	2/27/2014	Bison	F	adult
022714-14	6699	2/27/2014	Bison	F	adult
022714-15	6639	2/27/2014	Bison	F	adult
022714-16	6701	2/27/2014	Bison	F	adult
022714-17	6719	2/27/2014	Bison	F	juvenile
022714-18	6706	2/27/2014	Bison	F	adult
022714-19	6688	2/27/2014	Bison	F	adult
022714-20	6746	2/27/2014	Bison	F	adult
022714-21	6689	2/27/2014	Bison	F	adult
022714-22	6681	2/27/2014	Bison	F	adult
022714-23	6692	2/27/2014	Bison	F	adult
022714-24	6736	2/27/2014	Bison	F	adult
Y031	6614	2/27/2014	Bison	F	NA
030514-01	6793	3/5/2014	Bison	F	adult
030514-02	6768	3/5/2014	Bison	F	adult
030514-03	6766	3/5/2014	Bison	F	adult
030514-04	6797	3/5/2014	Bison	F	adult
030514-05	6779	3/5/2014	Bison	F	adult
030514-06	6762	3/5/2014	Bison	F	adult
030514-07	6754	3/5/2014	Bison	F	adult
030514-08	6794	3/5/2014	Bison	F	adult
030514-09	6764	3/5/2014	Bison	F	adult
030514-10	6757	3/5/2014	Bison	F	adult
030514-11	6760	3/5/2014	Bison	F	adult
030514-12	6805	3/5/2014	Bison	F	adult
030514-13	6778	3/5/2014	Bison	F	adult
030514-14	6792	3/5/2014	Bison	F	adult
030514-15	6775	3/5/2014	Bison	F	adult

From: [Nelson, Janell - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCluskey, Brian J - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: FYI -- Waiting for clarification on FOIA 12-1606
Date: Tuesday, February 21, 2012 1:25:43 PM

FYI

Please note, that we've asked that this request be nullified based on the notion that it was Dr. Clarke's calendar they wanted (since that's the one that was included in the previous FOIA response), but BFC actually did not specify Dr. Clarke's calendar, so everyone's is subject to whatever decision is made on this matter.

Janell

From: Bundy, Mildred O - APHIS
Sent: Tuesday, February 21, 2012 12:54 PM
To: Nelson, Janell - APHIS
Cc: FOIA Officer
Subject: RE: NEW FOIA: Search Memo - Case #: 2012-APHIS-01606-F - Correct Request

Janel: I will wait for FOIA to answer this....on both issues.

Thanks

From: Nelson, Janell - APHIS
Sent: Tuesday, February 21, 2012 12:51 PM
To: Bundy, Mildred O - APHIS
Cc: FOIA Officer
Subject: RE: NEW FOIA: Search Memo - Case #: 2012-APHIS-01606-F - Correct Request

Two questions arise from this request:

- 1) May we have a one-week extension? Dr. Clarke is on military duty this week and will not be available to provide a timely response.
- 2) Is it appropriate and legal for the agency to compel an employee to surrender copies of their personal appointment calendar? It is true that Dr. Clarke willingly provided a couple of pages from a previous year calendar because he had copies of those pages (and the notes he had made on that "handy" bit of paper) among the records requested in one of the dozen FOIAs this group has requested in the past few months.

Dr. Clarke should not be compelled to provide evidence of every personal appointment in his life to satisfy the zealous curiosity of this special interest group. Neither Dr. Clarke's military activities, his medical appointments, his anniversary plans, nor how much he spent on his lunch, should not fall subject to the scrutiny of this group or the general public. Since this group has posted to their website everything APHIS has disclosed through their numerous FOIA requests, it can be assumed that they would do the same with copies of Dr. Clarke's appointment calendar.

Janell R. Nelson

Staff Assistant

USDA APHIS VS Western Region | 2150 Centre Ave., Bldg B MS3E13 | Fort Collins, Colorado 80526 | 970-494-7400 | janell.r.nelson@aphis.usda.gov

From: Bundy, Mildred O - APHIS

Sent: Tuesday, February 21, 2012 7:21 AM

To: Nelson, Janell - APHIS

Cc: Bundy, Mildred O - APHIS

Subject: NEW FOIA: Search Memo - Case #: 2012-APHIS-01606-F - Correct Request

TO: VS-WR **REQUESTER:** GEIST

REQUEST #: FOIA-2012-APHIS-01606F **DUE TO FOIA:** 02/27/2012

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: MILDRED.BUNDY@aphis.usda.gov. If sending by mail, send to Mildred Bundy, USDA, APHIS, Room 4B02.9, 4700 Riverdale Road, Unit 50, Riverdale, MD 20737.

SEARCH START DATE: _____

Search time* (clerical): _____

Search time* (professional): _____

*Does not include photocopying time

Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
------	-------	------------------

_____	_____	_____
_____	_____	_____

Missing Document Explanation/Special Notes:

*****PLEASE NOTE: Agency records retention periods are affected by this FOIA/PA request. DO NOT DESTROY ORIGINALS for a minimum of 3 years. Please see APHIS Records Management Handbook: Inf 8 - Privacy Act Requests and Inf 9 - FOIA Requests.**

From: [McCollum, Matthew P. \(APHIS\)](#)
To: [Rhyan, Jack C. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [Frey, Rebecca K. \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#)
Subject: GnRH Conference call
Date: Tuesday, May 24, 2011 9:59:41 AM

Do we need to set another one up?

From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#)
Subject: GnRH protocol
Date: Thursday, May 10, 2012 2:50:00 PM
Attachments: [QA-1958 Rhyon Evaluation of GonaCon vaccine as a means of decreasing shedding of Brucella abortus in bison.pdf](#)
[ACUCBisonGonaConStudyfinal \(2\) with sigs.pdf](#)



Attached are the NWRC protocol and the Quarantine ACUC.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	Development of injectable and oral contraceptive technologies and their assessment for wildlife population and disease management

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: Jade C. Myers Date: 2/17/12

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:

Date _____ ☐ Res. Scientist ☐ Proj. Leader

☒ Research Scientist☐ Project Leader

☒ Visiting Scientist: NWRC Representative/Contact: LOWEN MILLER

☐ Student: NWRC Representative/Contact: _____

Concur: _____
NWRC Research Project Leader Jacob Ryan Date 2/17/12

Review and Processing: L. Heiner Date 2/21/12

Concur:
NWRC Assistant Director Mark E. Robin Date 2/22/12

Approved: _____ Date 2/22/12

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: <u>June 2, 2011</u>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement . Material Transfer agreements will be developed prior to material transfer
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?

If yes, attach Analytical Chemistry Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

4. Schedule

Proposed Experimental Start Date: April 15, 2012
 Proposed Experimental Termination Date: October 1, 2017
 Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

- | | |
|------|---|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer (<i>Odocoileus virginianus</i>): Pivotal target animal safety study |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (<i>Dama dama</i>) at Point Reyes National Seashore, California |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (<i>Cervus elaphus</i>) at Rocky Mountain National Park, Colorado |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (<i>Equus caballus</i>) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer |

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Null Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for histopathologic, bacteriologic, and molecular analysis. These will include: lymph nodes (bronchial, hepatic, internal iliac, popliteal, mandibular, parotid, prescapular, medial retropharyngeal, and supramammary), mammary gland tissue, spleen, lung, liver ovaries, uterus, cervix, adrenal glands, pituitary gland, and vaccination sites. Vaccinated cows will be euthanized in the chute via captive bolt and exsanguination or high-powered rifle. Alternatively they will be sedated, followed up with captive bolt and exsanguination. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d. Animal calving observation records
 - e. Pregnancy assessment records
- D. Final Report

13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400
E. Animal Care Cost	\$0	\$0	\$0					
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer

with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E. 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Yoder, C. A. and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title) _____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

A). Animal Information:

Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

B1) Rationale for involving animals:

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

B2) Rationale for numbers to be used: If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

B3) Rationale for appropriateness of the species to be used: Bison are the target species.

C) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

D) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

E) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

F) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

G) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

J) Dietary contaminant exposure NA

K) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

L) Animal pain or distress

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ____ Patrick Ryan Clarke _____

Date of Consultation: ____ 13 May 2011 _____

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

M) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_ See attached__

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyan has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

REGULATORY CONSIDERATIONS

Permits					
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <table style="width: 100%; border: none;"> <tr> <td style="border: none; width: 50%;">Permit(s) description</td> <td style="border: none; width: 30%;">Number</td> <td style="border: none; width: 20%;">Date</td> </tr> </table>	Permit(s) description	Number	Date
Permit(s) description	Number	Date			

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: _____Live animal work_____*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% shedding). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

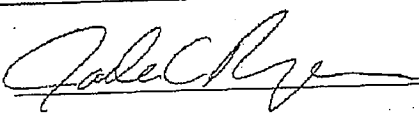
Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

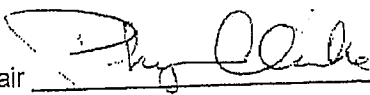
Miller, L. A., J. C. Rhyon, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

PART ONE: SIGNATURE PAGE

Study Director:  Date: 5/16/11

Concur:
IACUC Chair  Date 5/16/11

From: [Clarke, Patrick R. \(APHIS\)](#)
To: pj_white@nps.gov; [Rick Wallen](#)
Cc: [Frey, Rebecca K \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#)
Subject: Gona Con Study Bulls
Date: Wednesday, July 20, 2011 12:16:05 PM

PJ. Rick,

Of the four seronegative bulls that are being held at the Brogan facility to be used in the GonaCon study, three have now sero-converted and are considered reactors. From our experience with the Quarantine Feasibility Study, we anticipated that some animals would seroconvert after arriving at the facility so we are not surprised by this conversion. If you recall the outline of the GonaCon study, we will be using seropositive females, but cannot use any seropositive males for breeding.

We want to maximize the research potential of these bulls so instead of sending these animals straight to slaughter we thought we would send them to Jack Rhyan's facility. At Ft. Collins they would be held for serial blood/semen collection before being sent onward to slaughter at Double J in Pierce, CO.

We are not sure if the YNP permit would enable us to make this type of transfer of purpose/location. What are your thoughts?

Thanks,
Ryan

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#)
Subject: GonaCon Bison testing
Date: Tuesday, November 26, 2013 3:24:22 PM

Becky and I are looking at January 7-9, 2014 (Tuesday, Wednesday, Thursday) to work the GonaCon bison up here at Corwin Springs. I'm not sure if we'll need all three days, but certainly two. YNP has indicated they may be trapping and sending animals to slaughter as early as December. So the other factor in the mix this particular week is if the YNP trap is working we can also be getting "sniffer" samples and GonaCon cohort #2 animals.

Do these days work for youthe A Team.....???

I speak of you as the A Team now that they have split us apart and you have joined the special company of technical analysts, geniuses, savants, etc. in STAS....while Becky and I are relegated to the knuckle-draggers of District 5 in SPRS. I heard STAS people get every other Friday off as admin leave to either 1) reorganize their wine cellars or 2) play polo in the Hamptons. Is this true???

Have a good Thanksgiving,

The B Team

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Clarke, Patrick R. \(APHIS\)](#); [Rick Wallen](#)
Subject: GonaCon call Conference Details (MAY 31, 2011--02:00 PM MT--Conf# 5043744)
Date: Thursday, May 26, 2011 11:11:29 AM

Ok, we'll be talking Tuesday the 31st at 2:00.

Dial in number: (b) (6)

Participant passcode: (b) (6)

Thanks,
Matt

From: McCollum, Matthew P (APHIS)
Sent: Wednesday, May 25, 2011 3:15 PM
To: Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); Clarke, Patrick R. (APHIS); Rick Wallen
Subject: RE: GonaCon call?

So it sounds like everyone I've heard back from is available next Tuesday May 31 at 2:00 in the afternoon.

I'll go ahead and set up a call for then unless I hear otherwise.

I'll send out the call in numbers tomorrow.

Thanks,
Matt

From: Nol, Pauline (APHIS)
Sent: Tuesday, May 24, 2011 1:48 PM
To: Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); Clarke, Patrick R. (APHIS); Rick Wallen
Subject: RE: GonaCon call?

Sorry, I need to revise my last reply-I'm available Thursday and Friday afternoons and should be around most of next week. Pauline

From: Frey, Rebecca K (APHIS)
Sent: Tuesday, May 24, 2011 1:09 PM
To: McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Rick Wallen
Subject: Re: GonaCon call?

Thursday or Friday will work for me or Tuesday PM.
Becky Frey

From: Matt McCollum

Sent: 05/24/2011 04:48 PM GMT

To: Jack Rhyan; Jason Lombard; "Jenny_Powers@nps.gov" <Jenny_Powers@nps.gov>;
"Margaret_Wild@nps.gov" <Margaret_Wild@nps.gov>; Patrick Clarke; Pauline Nol; Rebecca Frey;
"Rick_Wallen@nps.gov" <Rick_Wallen@nps.gov>

Subject: GonaCon call?

Hi there,

It'd be good to get another call together to talk about where we are at with this. I've attached the final ACUC protocol that has been submitted.

Thursday the 26th is wide open for me at this point. Friday afternoon would work, or pretty much anytime next week. Let me know your availability please.

Thanks,

Matt

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Clarke, Patrick R. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Rick_Wallen@nps.gov](#)
Subject: GonaCon call?
Date: Tuesday, May 24, 2011 10:48:55 AM
Attachments: [ACUCBisonGonaConStudyfinal.pdf](#)

Hi there,

It'd be good to get another call together to talk about where we are at with this. I've attached the final ACUC protocol that has been submitted.

Thursday the 26th is wide open for me at this point. Friday afternoon would work, or pretty much anytime next week. Let me know your availability please.

Thanks,
Matt

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. <u> National Park Service </u> <u> YELL-2011-SCI-5892 </u> <u> May 10, 2011 </u> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> Permit(s) description Number Date </div>

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: Live animal work*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

on the occurrence of pregnancy and abortion or calving of infected animals. GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

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SIGNATURE PAGE

Study Director

Jade C. Ryan

Date

5/16/2011

Concur

IACUC Chair

Date

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: GonaCon Data Jan 8-9 2013---Vaginal Transmitter Frequencies Jan 25 2013
Date: Friday, January 25, 2013 3:06:28 PM
Attachments: [Jan 8-9 2013 testing data sheets.pdf](#)
[Jan-25-13 Vaginal Tranmitter Frequencies-GonaCon \(2 sheets\).xlsx](#)

I can pick up a signal from all but one of our 22 vaginal transmitters!

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

Jan 8, 2012

	A	B	C	D	E	F	G	H	I	J	K	L
1	BANGLE TAG	EARTAG	Sero-stat	Age/DOB	SEX	Old Eartag	Bled	Vag. Swab	Preg?	Deworm	Implant	Sniff?
2	Green 08	YNP930648	NEG	4, 2009	F		✓	✓	✓		.183✓	✓
3	Green 09	YNP930755	NEG	3, 2010	F		✓	✓	✓		.702✓	✓
4	Green 10	YNP930626	NEG	4, 2009	F		✓	✓	✓		.542✓	✓
5	Green 14	YNP930725	NEG	4, 2009	F		✓	✓	✓	.052✓	.621✓	✓
6	Green 15	YNP930634	NEG	3, 2010	F		✓	✓	✓		.132✓	✓
7	Red 03	YNP930689	POS	4, 2009	F		✓	✓	✓		.162✓	✓
8	Red 06	YNP930287	POS	3, 2010	F		✓	✓	✓		.572✓	✓
9	Red 07	YNP930773	POS	4, 2009	F		✓	✓	✓		.193✓	✓
10	Red 08	YNP930761	POS	4, 2009	F		✓	✓	✓			✓
11	Red 09	YNP930760	POS	2, 2011	F		✓	✓	open		.692✓	✓
12	Red 13	YNP930737	POS	3, 2010	F		✓	✓	✓	Warts	.333✓	✓
13	Red 15	YNP930706	POS	3, 2010	F		✓	✓	No : Alaskan?			✓
14	Red 16	YNP930684	POS	3, 2010	F		✓	✓	✓			✓
15	Red 17	YNP930588	POS	3, 2010	F		✓	✓	open		.752✓	✓
16	Red 18	YNP930776	POS	4, 2009	F		✓	✓	✓		.582✓	✓
17	Red 21	YNP930763	POS	4, 2009	F		✓	✓	✓		.301✓	✓
18	Red 22	YNP930673	POS	4, 2009	F		✓	✓	✓		.041✓	✓
19	Red 25	YNP930778	POS	4, 2009	F		✓	✓	✓		.633✓	✓
20	Red 30	YNP930568	POS	3, 2010	F		✓	✓	✓			✓
21												
22												
23												
24												
25												
26												
27												

Jan 9, 2012

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	BANGLE TAG	EARTAG	Sero-stat	Age/DOB	SEX	Old Eartag	BLED	Vag. Swab	Preg?	Disposition	Deworm	Implant	Sniff?
2	Green 02	YNP930702	NEG	3, 2010	F		✓	✓	✓	Frank's	✓	641✓	✓
3	Green 03	YNP930731	NEG	3, 2010	F		✓	✓	✓	Frank's	✓	551✓	✓
4	Green 04	YNP930625	NEG	4, 2009	F		✓	✓	open	Frank's	✓	—	✓
5	Green 06	YNP930754	NEG	3, 2010	F		✓	✓	open	Frank's	✓	—	✓
6	Green 17	YNP930627	NEG	4, 2009	F		✓	✓	✓	Frank's	✓	050✓	✓
7	Red 01	YNP930472	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
8	Red 02	YNP930705	SUS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
9	Red 04	YNP930759	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
10	Red 05	YNP930697	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
11	Red 11	YNP930777	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
12	Red 14	YNP930150	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
13	Red 19	YNP930762	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
14	Red 20	YNP930678	POS	4, 2009	F		✓	✓	✓	Frank's	✓	720✓	✓
15	Red 23	YNP930667	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
16	Red 24	YNP930636	POS	4, 2009	F		✓	✓	✓	Frank's	✓	801✓	✓
17	Red 26	YNP930202	POS	4, 2009	F		✓	✓	✓	Frank's	✓	662✓	✓
18	Red 27	YNP930454	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
19	Red 28	YNP930575	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
20	Red 29	YNP930406	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
21	Red 31	YNP930696	POS	3, 2010	F	Green 05	✓	✓	—	Frank's	✓	—	✓
22													
23													
24													
25													
26													
27													
28													

6392

6628

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Eckery, Douglas C - APHIS](#)
Cc: [Orahood, Darcy S - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Gonacon meeting?
Date: Wednesday, May 14, 2014 10:28:41 AM

Hi,

Darcy and I were just chatting in the hallway and it seems to me that a pow-wow (pow-ow?) might be in order to talk about Gonacon. What has been done and what can be done in future. Any interest? If so, who all should be there?

I'm pretty free next week except for Friday.

Thanks,

Matt McCollum

Wildlife Disease Biologist

USDA/APHIS/VS

Wildlife/Livestock Disease Investigations Team

4101 Laporte Ave

Fort Collins, CO 80521

(970)266-6233 Office

(b) (6) Mobile

From: [Nol, Pauline \(APHIS\)](#)
To: [Rhyen, Jack C \(APHIS\)](#)
Subject: GonaCon Montana protocol
Date: Monday, August 29, 2011 10:56:00 AM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 8 29 11.docx](#)



Jack,

I may have already sent you this version, but I can't remember and I'm fearing I keep dropping this ball. There are a few places that need some info that I cannot provide. They are tagged with comments. And then I think the rest is up to Chemistry to provide. I will send them this as well.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) X <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: June 2, 2011
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date: October 1, 2021

Anticipated Project Outcome: ☒ Manuscript
☒ Report
☐ Other: _____

Materials to be archived to close this activity: Raw data
Final Report

Description of Project and NWRC Activities and Participation: This study is not part of an NWRC Project.
NWRC's role in this study will be to provide GonaCon and to run ELISAs to determine anti-GnRH titers.

Comments:

Attachments:
(e.g. Material
Transfer Form,
IACUC approval,
etc.)

IACUC Protocol Approval

Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011

Proposed Experimental Termination Date: October 1, 2017

Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

QA-1112 GonaCon Immunocontraceptive Vaccine for White-tailed Deer (*Odocoileus virginianus*): Pivotal target animal safety study Pivotal field study of GonaCon

immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland

QA-1417 Pivotal field study of GonaCon immunocontraceptive vaccine for use in the

contraception of white-tailed deer in New Jersey Collection of ancillary data on GonaCon

QA-1445 Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (*Dama dama*) at Point Reyes National Seashore, California

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado

QA-1657 Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota

Chemical sterilization of black-tailed deer

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

Commented [jde1]: For the Experimental Use Permit (EUP), you will want to include a map showing the test site location and the layout of the pens (including size)

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

[Pregnancy and calving/abortion rates will be documented throughout the study.](#)

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. [Serology \(ELISA\) will also be conducted at NWRC to measure antibodies against GnRH.](#)

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses [of animals that have not been vaccinated with GonaCon](#) will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check

2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies

BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d.
 - e.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx	
A. Salary and Benefits				
B. Facilities (in addition to existing facility or space costs)				
C. Equipment				
D. Supplies				
E. Animal Care Costs				
F. Operating Costs (travel, misc. services, etc)				
TOTAL	\$0	\$0	\$0	

Commented [pn2]:

Commented [jde3]: Cost?

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E.⁵ 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. - J Wildl Dis. 34:582-9.

Yoder, C. A.⁵ and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☒ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☒ NEPA and ESA Appendix
 - ☒ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: (include appropriate title) _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart

Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. [The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes.](#) Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study.

However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

14) Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_ See attached____

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes

Commented [jde4]: You should be able to provide the names for contacts at a number of state and federal entities involved in bison management, particularly those involved in this study

Commented [jde5]: This is the person who manages the corrals where the bison will be kept

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRh/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided

to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): _____

Commented [jde6]: You need to talk to Doreen Griffin or Dave Goldade about this

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: GonaCon Montana: Just need some names for page 16 and I think we are good to go
Date: Tuesday, January 03, 2012 12:57:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft_12_29_11.docx](#)



Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6138

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	Development of injectable and oral contraceptive technologies and their assessment for wildlife population and disease management

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☒ Research Scientist☐ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: June 2, 2011
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
 Proposed Experimental Termination Date: October 1, 2017
 Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient

uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

- | | |
|------|---|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer (<i>Odocoileus virginianus</i>): Pivotal target animal safety study |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (<i>Dama dama</i>) at Point Reyes National Seashore, California |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (<i>Cervus elaphus</i>) at Rocky Mountain National Park, Colorado |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (<i>Equus caballus</i>) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer |

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Null Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for histopathologic, bacteriologic, and molecular analysis. These will include: lymph nodes (bronchial, hepatic, internal iliac, popliteal, mandibular, parotid, prescapular, medial retropharyngeal, and supramammary), mammary gland tissue, spleen, lung, liver ovaries, uterus, cervix, adrenal glands, pituitary gland, and vaccination sites. Vaccinated cows will be euthanized in the chute via captive bolt and exsanguination or high-powered rifle. Alternatively they will be sedated, followed up with captive bolt and exsanguination. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check; serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction

AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d. Animal calving observation records
 - e. Pregnancy assessment records
- D. Final Report

13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19	
A. Salary and Benef	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900	
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400	
E. Animal Care Cost	\$0	\$0	\$0						
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600	
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke Jack Rhyan is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E. 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Yoder, C. A. and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title) _____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

A). Animal Information:

Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

B1) Rationale for involving animals:

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

B2) Rationale for numbers to be used: If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

B3) Rationale for appropriateness of the species to be used: Bison are the target species.

C) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

D) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

E) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

F) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

G) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

J) Dietary contaminant exposure NA

K) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

L) Animal pain or distress

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ____ Patrick Ryan Clarke _____

Date of Consultation: ____ 13 May 2011 _____

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

M) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011 _See attached____

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes

Commented [jde1]: You should be able to provide the names for contacts at a number of state and federal entities involved in bison management, particularly those involved in this study

Commented [jde2]: This is the person who manages the corrals where the bison will be kept

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

From: [Nol, Pauline \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#)
Subject: GonaCon Protocol
Date: Tuesday, August 16, 2011 1:52:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 8 16 11.docx](#)



Jack,

Can you help with some names etc. for page 22 (NEPA appendix)? The other details on the Chemistry side of things should be dealt with on the Product Development side, don't you think?

P

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) X <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: June 2, 2011
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date: October 1, 2021

Anticipated Project Outcome: ☒ Manuscript
☒ Report
☐ Other: _____

Materials to be archived to close this activity: Raw data
Final Report

Description of Project and NWRC Activities and Participation: This study is not part of an NWRC Project.
NWRC's role in this study will be to provide GonaCon and to run ELISAs to determine anti-GnRH titers.

Comments:

Attachments:

(e.g. Material
Transfer Form,
IACUC approval,
etc.)

IACUC Protocol Approval

Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011

Proposed Experimental Termination Date: October 1, 2017

Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

QA-1112 GonaCon Immunocontraceptive Vaccine for White-tailed Deer (*Odocoileus virginianus*): Pivotal target animal safety study Pivotal field study of GonaCon

immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland

QA-1417 Pivotal field study of GonaCon immunocontraceptive vaccine for use in the

contraception of white-tailed deer in New Jersey Collection of ancillary data on GonaCon

QA-1445 Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (*Dama dama*) at Point Reyes National Seashore, California

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado

QA-1657 Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota

Chemical sterilization of black-tailed deer

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

Commented [jde1]: For the Experimental Use Permit (EUP), you will want to include a map showing the test site location and the layout of the pens (including size)

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Pregnancy and calving/abortion rates will be documented throughout the study.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check

Commented [jde2]: NWRC will conduct ELISA tests to determine anti-GnRH titers

EPA will want to see two measures of efficacy to prove GonaCon will work in bison. This study will actually have more than two measures: 1) pregnancy rates, 2) number of calves produced, 3) anti-GnRH titers

Commented [jde3]:

This should be pointed out in the EUP. I want EPA to know you intend to send the animals to slaughter at the end of the study

2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies

BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d.
 - e.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx	
A. Salary and Benefits				
B. Facilities (in addition to existing facility or space costs)				
C. Equipment				
D. Supplies				
E. Animal Care Costs				
F. Operating Costs (travel, misc. services, etc)				
TOTAL	\$0	\$0	\$0	

Commented [pn4]:

Commented [jde5]: Cost?

14. Human Health and Safety

HS004-00	Personal protective equipment
----------	-------------------------------

15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E.⁵ 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. - J Wildl Dis. 34:582-9.

Yoder, C. A.⁵ and L. A. Miller. 2010. Effect of GonaConTM vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☒ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☒ NEPA and ESA Appendix
 - ☒ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: (include appropriate title) _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart

Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. [The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes.](#) Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

14) Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

N. IACUC Approval

Date of IACUC Approval Letter: ACUC Protocol approved 5/17/2011_See attached

Commented [pn6]: By Montana IACUC-Name?

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes

Commented [jde7]: You should be able to provide the names for contacts at a number of state and federal entities involved in bison management, particularly those involved in this study

Commented [jde8]: This is the person who manages the corrals where the bison will be kept

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRh/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided

to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): _____

Commented [jde9]: You need to talk to Doreen Griffin or Dave Goldade about this

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [Clarke, Patrick R. \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: GonaCon serology testing
Date: Thursday, July 28, 2011 9:26:54 AM

It only took a little arm twisting to get Marty Zaluski to commit to paying for all the serology costs of the GonaCon study(for blood submitted to the Bozeman DOL lab).

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

From: [Clarke, Patrick R. - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: GonaCon Shopping List
Date: Thursday, February 02, 2012 4:24:44 PM

YNP says if the bison movement is right they are prepared to trap as early as Feb 13th.....the first animals would go to the GonaCon study. Do we have a "shopping list" of the animals we still need for the study (age ,sex, sero-status, etc.) that we can share with YNP?

Cheers,

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: [Nol, Pauline - APHIS](#)
To: [Miller, Lowell A - APHIS](#); [Orahood, Darcy S - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: GonaCon study bison-Montana
Date: Wednesday, March 28, 2012 1:48:00 PM



Hi Darcy and Lowell,

Our target date of vaccinating the bison up in Montana is April 15! Time has been flying for sure!

We would like 20 doses of 3000ug/syringe (3 ml) GonaCon by that time. Will that work with your schedules?

Thanks and let us know of any foreseeable problems or questions.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Matt McCollum](#)
To: [Jason E Lombard](#); [Jack C Rhyan](#); [Jenny Powers@nps.gov](#); [Margaret Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline.Nol@aphis.usda.gov](#); [Rebecca.K.Frey@aphis.usda.gov](#); [Rick Wallen@nps.gov](#)
Subject: GonaCon Study Call #2
Date: Tuesday, May 03, 2011 3:52:00 PM

Would it work for everyone to move our conference call back one hour from 10:00 Tuesday May 10 to 11:00? A conflict has come up for Jack at the 10:00 time.

Thanks,
Matt

From: [Nol, Pauline - APHIS](#)
To: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
Subject: Gonacon study protocol
Date: Friday, January 11, 2013 10:09:00 AM
Attachments: [ACUCBisonGonaConStudyfinal \(2\) with sigs.pdf](#)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

Permits								
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p>						
		<table border="1"> <thead> <tr> <th>Permit(s) description</th> <th>Number</th> <th>Date</th> </tr> </thead> <tbody> <tr> <td> </td> <td> </td> <td> </td> </tr> </tbody> </table>	Permit(s) description	Number	Date			
Permit(s) description	Number	Date						

Nature of the Collaboration:

☐ *Advisory Committee participation*

☒ *Manuscript/review article collaboration*

☐ *Training program requiring the use of animals*

☒ *Data analysis, interpretation and reporting*

☒ *Other: Live animal work*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

End Date: October 1, 2017

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyen et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% shedding). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

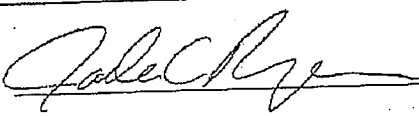
Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

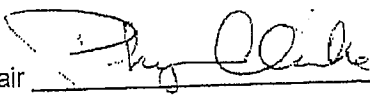
Miller, L. A., J. C. Rhyon, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

PART ONE: SIGNATURE PAGE

Study Director:  Date: 5/16/11

Concur: IACUC Chair  Date 5/16/11

From: [Matt McCollum](#)
To: [Margaret_Wild@nps.gov](#); [Jenny_Powers@nps.gov](#); [Rick_Wallen@nps.gov](#); [Jack C Rhyan](#);
[Pauline.Nol@aphis.usda.gov](#); [Patrick R Clarke](#); [Rebecca.K.Frey@aphis.usda.gov](#); [Jason E Lombard](#)
Subject: GonaCon Study
Date: Thursday, April 14, 2011 2:29:00 PM

Hi folks,

We are wondering if there is a time in the not too distant future that we could all get together on the phone and talk about the gonacon study. I can set up a conference call if we can define a time that'd work. How about the week of April 25th? Can we put together a call for Tuesday April 26 at 11:00? If that time does not work for you, is there another time later in the week that would? If there is anyone else that you think I should have included in this, please let me know.

Thank you,

Matt McCollum

Wildlife Biologist
USDA-APHIS-VS-WRO
National Wildlife Research Center
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 - Office
(b) (6) - Mobile
(970)266-6138 - Fax

"Whatever you are, be a good one." -Abraham Lincoln

From: [Rhyon, Jack C - APHIS](#)
To: [Stephens, Stephanie H - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: GonaCon study
Date: Thursday, November 03, 2011 11:48:32 AM
Attachments: [Tribal letter GonaConStudy.docx](#)

Stephanie,

Here it is. I'm ccing Pauline who will likely be able to improve the brief project writeup.

Jack



United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Washington, DC
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease shedding of Brucella abortus, the causative agent of brucellosis. We wanted to notify you of this potential project and changes to our animal disease regulations and are requesting your comments.

APHIS plans to publish an environmental assessment concerning this project soon. final rule amending requirements for the interstate movement of livestock and poultry in title 9, Code of Federal Regulations (9 CFR). Specifically, we are considering changes to section 71.20, approval of livestock facilities, and section 71.21, tissue and blood testing at slaughter. The proposed changes, which are summarized below, will increase our ability to safeguard livestock and poultry through early detection and reduced spread of foreign, emerging, and domestic program diseases. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. Some of these animals were captured last spring and the remainder will be captured in upcoming years. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years. Half of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept separate. Animals will be maintained and abortions and births monitored. Seronegative bison will be placed with the seropositive contracepted animals and with the seropositive non-contracepted animals to evaluate transmission. Following each parturition event, all bison will be examined for shedding of the organism.

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, animals that meet the requirements for quarantine will be placed on tribal or public lands.

Approval of Livestock Facilities (9 CFR 71.20)

Currently, to be an approved livestock market or to maintain approval, individuals legally responsible for the day-to-day operations of the livestock facility must meet certain conditions. Namely, they must sign an agreement entitled, "Approved Livestock Facility for Handling Livestock Pursuant to title 9 of the Code of Federal Regulations." In addition, they must keep records such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in, the facility. The records are required to be maintained for 2 years. The changes we are considering to the regulations would increase this recordkeeping requirement to 5 years.

Commented [DAR1]: if a proposed rule, say proposed



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1-800-877-8339

Tissue and Blood Testing at Slaughter (9 CFR 71.21)

Under 9 CFR 71.21, livestock or poultry moving interstate for slaughter or rendering can only be moved to a slaughtering or rendering establishment that has been approved and listed by the APHIS Administrator. For an establishment to be listed, the operator of the establishment must agree to a number of provisions, such as:

- Allowing APHIS and Food Safety and Inspection Service personnel, or APHIS contractors, access to the facility to take blood and tissue samples from animals at the facility
- Retaining individual identification of animals
- Providing office space with necessary furnishings, light, temperature control, and janitorial service

Under the current regulations, operators of slaughtering or rendering establishments are not required to sign an agreement, and there are no recordkeeping requirements for these establishments.

However, as amended in the draft final rule, operators of slaughtering and rendering establishments would be required to sign a listing agreement, if they move animals interstate. Owners or operators that do not move livestock or poultry interstate are not required to be listed. The agreement will show that operators agree to meet the requirements for listed slaughtering and rendering establishments. In the event of a disease outbreak, APHIS may need to collect samples at certain facilities. By having an agreement in place, it will be easier to detect and prevent the spread of foreign, emerging, or domestic animal diseases.

In addition, we are considering adding recordkeeping requirements. Owners and operators will be required to keep documents such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in the facility, for 5 years. Retaining these records, as well as those for approved livestock facilities, for 5 years will help us find potentially infected or exposed livestock or poultry more quickly and enable us to do a more in-depth traceback. Livestock owners will benefit from reductions in the time needed to find animals that have been exposed to disease, which may reduce the time needed for quarantines.

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project's potential changes. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at Terry.W.Clark@aphis.usda.gov or by telephone at (919) 855-7167.

Sincerely,

Commented [dcc2]: The current rule states that they must agree to allow us access, etc., in order to be listed by the Administrator so it is assumed they would agree in writing but the actual agreement is not in the current rule. I would change the wording to state that the agreement is not in the rule because they are already required to sign the agreement and many establishments have done so.

Commented [DAR3]: True?

Commented [DAR4]: True?

Commented [dcc5]: Yes

Commented [DAR6]: If we have proposed these changes, do we want to enclose the proposed rule for additional info?

Tribal Leader
Page 3

John R. Clifford
Deputy Administrator

From: [Jason E Lombard](#)
To: [Matt McCollum](#); [Rick Wallen@nps.gov](#)
Cc: [Jack C Rhyan](#); [Jenny Powers@nps.gov](#); [Margaret Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: GonaCon Study
Date: Friday, May 13, 2011 9:10:00 AM

Hello,

I had the opportunity to discuss the study with 2 statisticians from CEAH yesterday and wanted to share with you what we discussed.

If we use seroconversion of sentinels as the outcome, we have to use pasture as the experimental unit and with only 4 pastures which we had talked about during the call, we have no power to detect any differences in seroconversion. A better option would be to measure shedding at the individual animal level (primarily seropositives) and with multiple samples per animal per sampling date and multiple dates, we will have repeated measures on each animal. We can also sample seronegatives for shedding and maybe will be able to detect shedding prior to seroconversion which I understand hasn't been fully investigated in bison. I talked with Jack and Matt yesterday and they were thinking about having 16-18 seropositives and 4 seronegatives per pasture which would give us plenty of animals to get a handle on shedding. I am not sure how you quantify shedding in animals that have aborted, had a stillborn or a normal calf where you have potentially large amounts of bacteria compared with animals that don't calve but I'm sure we can figure something out.

We had discussed replacing the control sentinels after all had seroconverted but I'm not sure that is necessary unless we want to evaluate shedding in more non-vaccinated animals, besides we are only talking about 4 bison at this point. It might be more interesting to see if shedding increases as the proportion of seropositives in a pen increases, although with only 4 sentinels per pasture, 80% will be seropositive from the start. We could also look at it from a survival analysis or time to event perspective. When all the animals in the control pastures have seroconverted, we probably don't need to keep following them.

Cheers!
Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

From: [Stephens, Stephanie H - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: GonaCon Tribal Letter
Date: Monday, November 07, 2011 8:50:13 AM
Attachments: [Tribal letter GonaCon 11.7.11.docx](#)
Importance: High

Hi Jack and Pauline-

I've done some revisions to the draft tribal letter for the GonaCon study in Montana. There were a some comments internally from ERAS that the letter had some terms in it that might be better explained in simpler language. So the revisions I made have attempted to address this issue.

Can you please review the attached draft letter and let me know if it looks ok to you? If I've garbled anything important, please also correct me on that! Once you've commented, I'll get this letter back to Terry Clark so he can finalize and send it out.

Thanks,

Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134



United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Washington, DC
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease shedding exposure to of *Brucella abortus*, the causative agent of bacteria that can cause brucellosis. APHIS plans to publish an environmental assessment concerning this project soon. We wanted to notify you of this potential project and are requesting your comments.

One significant way that brucellosis can be spread between infected and uninfected bison happens when infected animals give birth. The materials associated with giving birth contain *Brucella abortus*, and uninfected bison often become exposed to the infected material. We wanted to notify you of this potential project and are requesting your comments. The study that APHIS wants to conduct will investigate one way to decrease the potential for this exposure to take place by preventing infected bison from giving birth.

APHIS plans to publish an environmental assessment concerning this project soon. Some of the animals that will be used in the study were captured last spring and the remainder will be captured in upcoming years. Blood samples will be collected from captured bison to test to see if there is evidence of brucellosis infection. Bison that test positive for the presence of brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. Some of these animals were captured last spring and the remainder will be captured in upcoming years. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years.

In the proposed study, Hhalf of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the GonaCon® vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile from the vaccine, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept in separate areas during the study. Animals will be maintained-cared for throughout the study and abortions and births will be monitored. Seronegative bison will be placed with the seropositive GonaCon®-vaccinated ~~contracepted~~ animals and with the seropositive non-~~contracepted~~ GonaCon®-vaccinated animals to evaluate transmission of brucellosis. Following each birthing event, all bison will be examined for shedding of *Brucella* bacteria to see if they have infected materials that are capable of transmitting *Brucella abortus* to other bison.



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Tribal Leader
Page 2

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, ~~Brucella negative~~~~Non-infected~~ animals that have tested negative for brucellosis that also meet the requirements for previously-established quarantine use will be placed on tribal or public lands.

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at Terry.W.Clark@aphis.usda.gov or by telephone at (919) 855-7167.

Sincerely,

John R. Clifford
Deputy Administrator

From: [Clarke, Patrick R. - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: IACUC signatures VOC and GonaCon
Date: Tuesday, January 28, 2014 1:53:30 PM
Attachments: [GonaCon Protocol renewal Dec 2013 signed.pdf](#)
[VOC Protocol renewal Dec 2013 signed \(1\).pdf](#)
[VOC Protocol renewal Dec 2013 signed \(2\).pdf](#)

Pauline,

Between the 3 scans attached we have all the required signatures form all the IACUC members for both studies. Sorry about the delay.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

Study Protocol: J Rhyan

Animal usage (please complete the following box):

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved FOR TOTAL PROJECT on current approval notification plus any subsequent amendments	104			
2. Number of animals used during first IACUC approval year	40			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	40			
4. Number of animals used during third approval year	42			

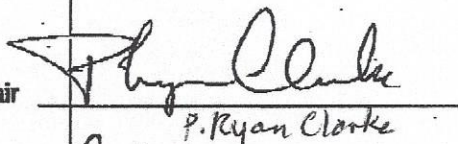
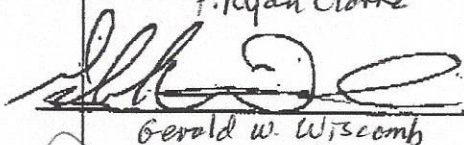
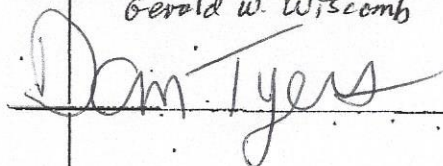
Note: Additional animals (up to 62) will be collected in winter/spring 2014 to replicate the study as described in the original protocol.

Study Director

Date 12/12/13

Concur

IACUC Chair


P. Ryan ClarkeDate 12/16/13
Gerald W. Wiscomb12/17/13
Dan Tyers1/25/14

Animal usage (please complete the following box):

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	100			
2. Number of animals used during first IACUC approval year	38			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	20			
4. Number of animals used during third approval year (or to be used if in future)	60			

Note: the 20 animals used in year two were repeat collections of animals also sampled in year one.

Study Director

Jacob Rhyan

Date 12/18/13

Concur

IACUC Chair

Phyllis Clarke

Date 1/2/14

IACUC
member

Dan Tyers

Date 1/25/14

IACUC
member

Date _____

Study Protocol: J Rhyon

Animal usage (please complete the following box);

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	100			
2. Number of animals used during first IACUC approval year	38			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	20			
4. Number of animals used during third approval year (or to be used if in future)	60			



Note: the 20 animals used in year two were repeat collections of animals also sampled in year one.

Study Director

Date 12/18/13

Concur

IACUC Chair


P. Ryan ClarkeDate 1/2/14IACUC
member
Jerry WiscombDate 1/6/14IACUC
member

Date _____

From: [Jack C. Rhyan](#)
To: [Pauline Nol](#); [Matt McCollum](#); [Patrick R. Clarke](#); [Rebecca K. Frey](#)
Subject: Immunocontraceptive project
Date: Tuesday, October 12, 2010 3:52:00 PM
Attachments: [ImmunocontBisonProject_10-12.doc](#)

OK folks. Here tis. Please see what you think. Get out your red pencils. Pauline and I struggled a bunch with what to do with offspring. Do we keep them in with the adults for the entire study? Do we separate them prior to calving? The easiest thing would be to kill em before the next calving but what is the best science? I'd like to visit with yall tomorrow after you've had some ponderin' time. When's good? 9 am?

Jack

(See attached file: ImmunocontBisonProject_10-12.doc)

Proposed Project:

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites

2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

This general research plan will be followed. A total of 40 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately half seronegative and half seropositive) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and bi-annually thereafter. Bulls will be maintained separately and monitored by serology. In spring 2012, animals will be relocated into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive GonaCon™ vaccine and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female sentinel bison will be fitted with proximity collars programmed to record proximity to one another and to transmitters on vaginal implants. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). During that period, calving, the occurrence of abortions, and serology in the groups will be monitored. In February each year, animals will be pregnancy tested and pregnant animals fitted with vaginal transmitters. Transmitters will alert investigators to abortion or calving events and record exposure of sentinel animals. Animals will be tested by serology in February and in summer following calving. At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. Offspring from the study will be monitored by serology twice a year throughout the study. Offspring that remain or become positive for *B. abortus* by serology after weaning will be euthanized and necropsied. Offspring that remain serologically negative for brucellosis will be utilized for bison genetics conservation.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate.

Commented [rkf1]: Three years from when? After injection; or after 3 possible births. In other words...if we were to get animals in 2011, when would they be necropsied? Is there any need to keep them more than 3 years?

Commented [rkf2]: Had to read twice to comprehend.....all animals from original capture only?

Spring 2012 – Place groups in pastures for study.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions.

Summer 2015 – Euthanize, necropsy and culture study animals; utilize calves for genetic conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

From: [Jack C. Rhyan](#)
To: [Pauline Nol](#)
Subject: Immunocontraceptive study
Date: Tuesday, October 12, 2010 12:13:00 PM
Attachments: [ImmunocontBisonProject_10-12.doc](#)

(See attached file: ImmunocontBisonProject_10-12.doc)

Proposed Project:

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

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Commented [rkf1]: Three years from when? After injection; or after 3 possible births. In other words...if we were to get animals in 2011, when would they be necropsied? Is there any need to keep them more than 3 years?

Commented [rkf2]: Had to read twice to comprehend.....all animals from original capture only?

Time line:

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3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

From: [Nol, Pauline - APHIS](#)
To: [Stahl, Randal S - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: Info on Bison
Date: Thursday, September 19, 2013 12:28:00 PM
Attachments: [Montana Brucella Bison Herd January 2013.docx](#)

Hi Randy,

I think this is what you need on the bison except we still need to fill in the details on serology. Could that be broken down into weak or strong positive? 1 or 2?

FYI Sites 1 and 2 are about 2 miles apart I think.

Did you analyze any bison from our facility here? I can't remember.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

Bison Brucella VOC Study 2013
Breath Samples from Bison Participating
in Gonacon Study
Sampling Date: January 8-9, 2013

ID	Brucella Status	Treatment Group	Site	GnRH titer	Pregnancy Status	Intensity of reaction on Serology
R08	Postive	control	1	0	Pos	
R09	Postive	control	1	0	Neg	
R13	Postive	control	1	0	Pos	
R15	Postive	control	1	0	Neg	
R19	Postive	vax	2	128	Neg	
R20	Postive	vax	2	128	Pos	
R22	Postive	control	1	0	Pos	
R24	Postive	vax	2	0	Pos	
R27	Postive	vax	2	128	Neg	
R28	Postive	vax	2	128	Neg	
G2	neg	Sentinel	2	0	Pos	NA
G3	neg	Sentinel	2	0	Pos	NA
G4	neg	Sentinel	2	0	Neg	NA
G6	neg	Sentinel	2	0	Neg	NA
G8	neg	Sentinel	1	0	Pos	NA
G9	neg	Sentinel	1	0	Pos	NA
G10	neg	Sentinel	1	0	Pos	NA
G14	neg	Sentinel	1	0	Pos	NA
G15	neg	Sentinel	1	0	Pos	NA

Site 1 =Slip n Slide

Site 2 = Rigler's

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: info to ponder
Date: Thursday, January 02, 2014 10:06:29 AM

DATE	ID	EVENT	SERO-Cow	SERO-calf	Culture	Tissues
31-Jan	Red 03	Abortion	Pos	n/a	Pos	milk, vag, implant
15-Feb	Green 15	Abortion	Pos	n/a	Neg	
10-Apr	Red 16	Abortion	Pos	n/a	Pos	milk, vag, implant, placenta, feces, fetus
19-Apr	Green 09	wk calf	Pos	n/a	Pos	milk, placenta, implant, vag,
19-Apr	Red 21	calf	Pos	Pos	Pos	vag, placenta
28-Apr	Green 10	abortion	Pos	n/a	Pos	Abortion, milk, vag, placenta, feces
29-Apr	Green 14	calf	Neg	Neg	Neg	
7-May	Red 13	calf	Pos	Pos	Pos	Vag, exudate
8-May	Red 25	calf	Pos	Pos	Neg	
8-May	Green 08	calf	Neg	Neg	Neg	
10-May	Red 22	calf	Pos	Pos	Neg	
14-May	Red 07	calf	Susp	Pos	Neg	
14-May	Red 08	calf	Pos	Pos	Neg	
16-May	Red 20	calf	Pos	Pos	Neg	
16-May	Red 18	calf	Pos	Pos	Neg	
19-May	Red 30	calf	Pos	Pos	Neg	
23-May	Green 17	calf	Neg	Neg	Neg	
24-May	Red 26	calf	Pos	Pos	Neg	
5-Jun	Green 02	calf	Neg	Neg	Neg	
11-Jun	Green 03	calf	Neg	Neg	Neg	
2-Jul	Red 24	calf	Pos	Neg	Neg	

A chart with the calving info. We already decided that you would take the calves from Red 21 and Red 13. Any others you want??? Don't forget to bring your trailer!!!!

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [McCollum, Matthew P - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: January 16th, 2015 GnRH bison serum
Date: Thursday, March 05, 2015 11:04:29 AM

We have serum samples here from bison numbers R03, R06, R07, R08, R09, R13, R16, R17, R18, R20, R21, R22, R24, R25, R26, G08, G09, G10, G14, G15,

Matt McCollum

Wildlife Livestock Disease Investigations Team (WiLDIT)
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
(970)266-6233 Office
(b) (6) Mobile

From: [Nol, Pauline - APHIS](#)
To: [Stahl, Randal S - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: Locations of bison
Date: Friday, January 25, 2013 3:28:00 PM
Attachments: [Bison Stats for VOCs MT Gonacon Study.xlsx](#)

Hi Randy,

Here is the breakdown regarding site. Vaccine status may also influence the outcome.

Thanks Randy! And have a great weekend!

Pauline

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-Western Region

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

Montana: Gonacon Bison

ID	Treatment	Site
R08	control	1
R09	control	1
R13	control	1
R15	control	1
R19	vax	2
R20	vax	2
R22	control	1
R24	vax	2
R27	vax	2
R28	vax	2
G2	neg	2
G3	neg	2
G4	neg	2
G6	neg	2
G8	neg	1
G9	neg	1
G10	neg	1
G14	neg	1
G15	neg	1

Site 1 =Slip n Slide

Site 2 = Rigler's

From: Jenny_Powers@nps.gov
To: pauline.nol@aphis.usda.gov
Cc: Margaret_Wild@nps.gov
Date: Wednesday, March 09, 2011 10:16:00 AM
Attachments: [Rhyan Immunocontraception Study Plan_rlw review brmd.doc](#)

Hi Pauline,

Here are the comments from Margaret and I for you to work with when responding to comments.

Jenny

Jenny Powers, DVM
Wildlife Veterinarian
National Park Service
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525

Phone: (970) 267-2122

Cell: (b) (6)

Fax: (970) 225-3585

----- Forwarded by Jenny Powers/FTCOLLINS/NPS on 03/09/2011 10:13 AM -----

Rick
Wallen/YELL/NPS

To
03/04/2011 05:06 PM Jack.C.Rhyan@aphis.usda.gov
cc

Jenny Powers/FTCOLLINS/NPS@NPS
Subject

(See attached file: Rhyan Immunocontraception Study Plan_rlw review brmd.doc)

Jack: Jenny has returned my communication and accepted my proposal for her to participate on the immunocontraception study. I have talked with our research permitting coordinator and the advice she provided was to complete the study plan review and when the study plan is tightened up then submit it to her for a permit. She will have to send the proposal out for peer review and getting that process nailed down will allow the permitting

process to move rather quickly.

Jenny mentioned that she had contacted you and talked. I agree with the perspective that Jenny and Margaret have conveyed in their review that some advice from a biometrician will help ensure that the results of the proposal will be more credible. The power to detect a response is important as well as the identification of the actual response variable that are most important.

What are your thoughts on how to seek advise on study design from a statistician and when a revised study proposal can be turned around for submission to the research permit process. The research permitting group meets once per month so I will find out when they meet in March and if there is room to be on the agenda to present the proposal this month.

RW

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing ~~transmissions~~ shedding of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria ~~occurrence of following~~ pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission the shedding~~ of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* ~~transmissions~~ shedding in a bison herd

Commented [MAW1]: Transmission is not addressed here. Change to "potential for transmission" or "shedding"

Commented [MAW2]: Generally, the concept is unique and interesting. Proposal requires some more critical review and statistical input prior to implementation.

Commented [MAW3]: Then is the sample size proposed sufficient to address this question?

Commented [MAW4]: See comment on title

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Commented [MAW5]: How will you determine colonization? Is this once 3 yr post-vaccination? How much natural variation would you expect to see?

Commented [MAW6]: Excellent point. Study should continue after contraceptive effects have been lost.

3. Determine the effect of immune system stimulation via vaccination with GonaCon adjuvant on brucella titers and shedding.

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Indent at: 0.5"

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites.
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison, looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Commented [MAW7]: Granted the sample size is too small to do this, but it is a very important point. What is the potential for confounding effects of local inflammatory response from the adjuvant. Without adjuvant vaccinated controls, this can't be determined.

Commented [MAW8]: This portion of the study is not fully developed enough to know whether this objective could be met

Commented [MAW9]: Power?

Commented [MAW10]: Power?

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive – 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated.

Commented [MAW11]: How was this n selected? Will it allow adequate power based on expected outcomes?

Commented [MAW12]: What if the adjuvant has an effect? Lack of a group of adjuvant treated will result in confounding of results. Are results of "vaccination" from lack of reproduction or could they be confounded with immune stimulation? This design issue needs to be addressed before implementing this project.

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be ~~used~~ made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Commented [r13]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r14]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r15]: Why not get the culture done as soon as possible?

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined, preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector
↳ Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting? I'm not sure if this is answering a behavioral question (do bison investigate aborted fetuses) or a disease question (how often do aborted fetuses initiate seroconversion).
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy whether they would have an infectious live birth or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

Commented [jgp16]: Would be helpful to have a table of response variables, estimated variation expected between treatment groups, and what question they are meant to answer.

Commented [jgp17]: Is this measured through differences in proportion of seroconversion between the pastures, proportion culture positive, proportion with culture positive abortions, or ?? If using seroconversion as the marker what proportion of seroconversion would you expect on a yearly basis with exposure to seropositive females?

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Commented [jgp18]: Does this mean that we expect all animals to be seronegative at the end of the study or that no new ones will have seroconverted. This seems like it is directly related to the first outcome.

Commented [MAW19]: Are they infected or seropositive?

Commented [MAW20]: How will you do this and is power sufficient?

Commented [jgp21]: How do you rule out in-utero transmission?

Commented [MAW22]: This, like many of the other outcomes, seem to be overstated a bit. Some insights will no doubt be gained, but would like to see more evidence that data will actually allow "determination" of these factors.

Commented [MAW23]: Power? Pretty small sample of bulls, particularly if not all are actively breeding.

From: [Nol. Pauline - APHIS](#)
To: [Nol. Pauline - APHIS](#)
Date: Tuesday, February 21, 2012 11:31:58 AM
Attachments: [cofcwnadscan20030703020751.pdf](#)

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

Final ACUC protocol
5/23/11

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____ Permit(s) description _____ Number _____ Date _____

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: _____Live animal work_____*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL**1. Key Personnel**

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% ~~abortion~~ ^{shedding}). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

- 1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult
- 2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.
- 3) Rationale for appropriateness of the species to be used: Bison are the target species.
- 4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.
- 6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.
- 8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Attn: Jack K Ryan

Page 9 of 9

Study Protocol

GonaCon-in-bison

PART ONE: SIGNATURE PAGE

Study Director: [Signature]

Date: 5/16/11

Concur:
IACUC Chair [Signature] Date 5/16/11

From: [Eisemann, John D. \(APHIS\)](#)
To: [Fagerstone, Kathleen A. \(APHIS\)](#); [Rhyan, Jack C. \(APHIS\)](#); [Miller, Lowell A. \(APHIS\)](#); [Stephens, Stephanie H. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: Meeting to discuss the Bison Study
Date: Friday, June 03, 2011 10:46:29 AM

Jack and Kathy just set up a meeting at 2:00 pm (MT) to discuss the bison study. There are some important registration considerations that need to be discussed before the study planning goes too far. Hope you can make it. It will be in the conference room by my office. Stephanie, I will call you if you are available.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: [Jack C Rhyan](#)
To: [Kathleen A Fagerstone](#); [Lowell A Miller/CO/APHIS/USDA](#); [John D Eisemann/CO/APHIS/USDA](#)
Cc: [Pauline Nol](#); [Matt McCollum](#)
Subject: Monday meeting on Bison contraception project
Date: Friday, December 10, 2010 2:56:00 PM

Just confirming 10 am in products conf room
Jack

From: [Wehtje, Morgan E - APHIS](#)
To: [Mora, Darcy - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: MT Bison serum
Date: Thursday, October 27, 2016 2:41:14 PM

Darcy ,

The missing serum vials are in our UC in the VS freezer room (next door to the Gonacon room). They are on the top shelf of the freezer. The 2013 vials are large so maybe you just want to aliquot off what you need. There are not complete sets for each year. R08 is missing a 2013, Red15 , 30 and 23 are missing 2015 and Red 32 and higher do not have 2012 or 2013 draws. If you need anything else let me know.

Thanks

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: [Wehtje, Morgan E - APHIS](#)
To: [Mora, Darcy - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: MT Bison serum
Date: Thursday, October 27, 2016 2:41:14 PM

Darcy ,

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Thanks

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: MTFWP Scientific Collector's Permit
Date: Thursday, February 27, 2014 12:21:19 PM
Attachments: [GonaconStudy 2014 MTFWPSciColPermit.pdf](#)

MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS

Wildlife Bureau

P. O. Box 200701 · Helena, MT 59620-0701 · (406) 444-2612

SCIENTIFIC COLLECTOR'S PERMIT

Permit # 2014-022

IACUC # USDA APHIS (5/2014)

Fee: No Fee

Federal Permit #: NA ()

Date Issued: 1/1/2014

Permitted Activities: Scientific Collection

Date Expires: 12/31/2014

Multiple Year Permit (see note in conditions)

Permit issued to: Jack Rhyan

Address: USDA APHIS Veterinary Services
4101 Laporte Ave
Fort Collins, CO 80521

Phone number: 970-266-6140

Email: jack.e.rhyan@aphis.usda.gov

Associated with: USDA APHIS Veterinary Services

A report of activities conducted under the provisions of this permit must be sent to Montana Fish, Wildlife and Parks, Attn: Wildlife Bureau, POB 200701, Helena, MT 59620-0701 by December 31 annually. The report should list the number of animals handled, including species, date, location (GPS location in UTM coordinates or latitude-longitude if possible; or the legal description in Township, Range, Section and quarter section; otherwise a detailed description of location), other known biological information (sex, age, etc.) and/or cause of death if known. This information will be used for administrative purposes, and to supplement location information housed in the Montana Natural Heritage Program on species.

Subpermittees:

Dr. Pauline Nol , John Treanor , Rick Wallin , Chris Geremia , Becky Frey , Doug Blanton , Matt McCollum , Dr. Ryan Clarke

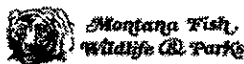
Copies of this permit must be in possession while engaged in activities.

This permit is not transferable.

Montana Fish, Wildlife & Parks

Ken McDonald

Administrator, Wildlife Division



Permit# 2014-022 Page 1 of 3

CC: Howard Burt
Justin Gude

MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS

Wildlife Bureau

P. O. Box 200701 · Helena, MT 59620-0701 · (406) 444-2612

SCIENTIFIC COLLECTOR'S PERMIT

Permit # 2014-022

Permit Conditions:

Study period: 2014

Study area: FWP region 3

Authorized Collection: Authorized to collect not more than 108 bison between January 2012 and May 2014 and transport them from Yellowstone National Park to the bison quarantine facility for Gona Con Trial.

Authorized to possess those animals and any subsequent offspring through 2019.

By end of study, seropositive animals shall be euthanized and useable meat donated to food banks. Disposition of seronegative animals including seronegative offspring shall be determined by MFWP.

Permit Expiration Date: This work has been approved for the life of this project. However, an updated permit must be issued annually and a current year permit must be in possession of the applicant to conduct work. To receive updated permits each year prior to the expiration date please notify MFWP in writing (email or hard copy) of your intent to continue work when submitting your annual report. Please also notify MFWP of any changes to subpermittees, methodology, or project objectives. MFWP reserves the right to request a new application and project proposal in the case of significant changes to methodology or project objectives.

Requests to continue work through an updated permit can be made prior to annual report submission for work that is ongoing during the months of December and January.

Copies of this permit must be in possession while engaged in activities.

This permit is not transferable.



Permit# 2014-022 Page 2 of 3

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Justin Gude

MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS

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SCIENTIFIC COLLECTOR'S PERMIT

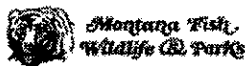
Permit # 2014-022

Other Relevant Montana Code Annotated and Administrative Rules of Montana:

MCA 87.2.806- MCA 87.5.109-

**Copies of this permit must be in possession while
engaged in activities.**

This permit is not transferable.



Permit# 2014-022 Page 3 of 3

CC: Howard Burt
 Justin Gude

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#); [Bens, Catherine M - APHIS](#); [Greiner, Steven J - APHIS](#)
Subject: New draft of QA1858
Date: Friday, February 17, 2012 9:24:00 AM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 2 17 12.docx](#)



I'll have a new signature page for you today.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	Development of injectable and oral contraceptive technologies and their assessment for wildlife population and disease management

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:

_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader

☒ Research Scientist

☐ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact: _____

☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: <u>June 2, 2011</u>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement . Material Transfer agreements will be developed prior to material transfer
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?

		If yes, attach Analytical Chemistry Appendix .
--	--	---

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Ryan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

4. Schedule

Proposed Experimental Start Date: April 15, 2012
 Proposed Experimental Termination Date: October 1, 2017
 Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

- | | |
|------|---|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer (<i>Odocoileus virginianus</i>): Pivotal target animal safety study |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (<i>Dama dama</i>) at Point Reyes National Seashore, California |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (<i>Cervus elaphus</i>) at Rocky Mountain National Park, Colorado |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (<i>Equus caballus</i>) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer |

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Null Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for histopathologic, bacteriologic, and molecular analysis. These will include: lymph nodes (bronchial, hepatic, internal iliac, popliteal, mandibular, parotid, prescapular, medial retropharyngeal, and supramammary), mammary gland tissue, spleen, lung, liver ovaries, uterus, cervix, adrenal glands, pituitary gland, and vaccination sites. Vaccinated cows will be euthanized in the chute via captive bolt and exsanguination or high-powered rifle. Alternatively they will be sedated, followed up with captive bolt and exsanguination. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyen et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d. Animal calving observation records
 - e. Pregnancy assessment records
- D. Final Report

13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400
E. Animal Care Cos	\$0	\$0	\$0					
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. *Am J Reprod Immunol.* 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. *Am J Reprod Immunol.* 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. *J Wildl Dis.* 40: 725-30

Rankin, J. E. 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. *Vet Rec.* 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. *J Wildl Dis.* 34:582-9.

Yoder, C. A. and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. *Vaccine.* 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title) _____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

A). Animal Information:

Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

B1) Rationale for involving animals:

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

B2) Rationale for numbers to be used: If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

B3) Rationale for appropriateness of the species to be used: Bison are the target species.

C) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

D) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

E) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

F) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

G) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

J) Dietary contaminant exposure NA

K) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

L) Animal pain or distress

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ____ Patrick Ryan Clarke _____

Date of Consultation: ____ 13 May 2011 _____

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

M) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_ See attached__

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyon has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

From: [Bundy, Mildred O - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Quance, Christine R - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#); [Bundy, Mildred O - APHIS](#); [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Subject: New FOIA Search Memo - 2012-APHIS-01161-F
Date: Tuesday, February 21, 2012 10:59:03 AM
Attachments: [12-01161.pdf](#)
[image001.png](#)

Hey Jack: I am attaching the original request. Thank you for working on this! I appreciate it.

I won't let them "haul" you away.

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 9:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS (Mildred.Bundy@aphis.usda.gov)
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR **REQUESTER:** GEIST
REQUEST #: 2012-APHIS-01161F **DUE TO FOIA:** 1/18/12

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: mildred.bundy@aphis.usda.gov, if sending by mail, send to USDA, APHIS, MILDRED BUNDY, 4700 Riverdale Road, Riverdale, MD 20737.

SEARCH START DATE:

Search time* (clerical): _____

Search time* (professional): _____

***Does not include photocopying time:** _____

Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
------	-------	------------------

Missing Document Explanation/Special Notes:

12-01161



Darrell Geist
<z@wildrockies.org>
01/06/2012 02:19 PM

To FOIA Officer/MD/APHIS/USDA
cc <z@wildrockies.org>, Patrick R Clarke/MT/APHIS/USDA
bcc
Subject JANUARY 6 2012 FREEDOM OF INFORMATION ACT
REQUEST

1 attachment



P1D1278DE 6 1 2.png

FOIA Request # 12-01161
Date Rec'd 1/6/2012
Date Due 1/6/2012
Assigned to Robbie
Category All other
Search VS



BUFFALO FIELD CAMPAIGN

P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
(406) 646-0070 PHONE (406) 646-0071 FAX
<http://www.buffalofieldcampaign.org>
buffalo@wildrockies.org

January 6, 2012

Tonya Woods, FOIA/PA Officer
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 50
Riverdale, MD 20737-1232
Tel. 301-734-5267
Fax 301-734-5941
Email: FOIA.Officer@aphis.usda.gov

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Ms. Woods:

Pursuant to the federal Freedom of Information Act (5 U.S.C. 552 et. seq.), Buffalo Field Campaign is filing this request for information.

Buffalo Field Campaign is a 501(c) (3) non-profit, public interest, grassroots media-based organization, which provides news reports directly to thousands of supporters which include concerned American citizens, and people from around the globe, as well as to regional, national and international media.

We would prefer an electronic copy of this information on CD but we would be happy to get a paper copy of anything that is not available electronically.

We request the following documentation from USDA APHIS:

1. Brucella Genotyping Reports (final, preliminary, draft) generated by APHIS during calendar years 2010 and 2011 for incidents or suspected incidents of *brucella abortus* infection in elk, bison and cattle in Montana, Idaho, and Wyoming.

As you know, the Freedom of Information Act (FOIA) provides that if portions of a document are exempt from release, the remainder must be segregated and disclosed. We expect to receive all non-exempt portions of the documents that we have requested, and ask that you justify any deletions by reference to specific exemptions allowed under the FOI Act. The Buffalo Field Campaign reserves the right to appeal a decision to withhold any materials.

We hereby request a fee waiver for all search and duplication fees under the FOIA regulations [5 U.S.C. Sec. 552 (a) (4) (A) and 36 CFR 2.19(c) (1)]. The information requested will benefit the citizens of the United States and is for the purpose of public education and to encourage public debate on important policy issues. The requested information will be made available to the public through Buffalo Field Campaign's central office and/or our website.

Information available through the office and website is used in press conferences and releases, television and radio interviews, and regional and national publications, and reaches a significant number of individuals nationwide, including through the following news sources: New York Times, Los Angeles Times, Washington Post, CNN, CBS, ABC, NBC, Headline News, London Times, UK Guardian, Japanese and German TV, National Geographic, PBS, Associated Press (nationally syndicated), Reuters (internationally syndicated), Planet Green Discovery Channel, Examiner, Indian Country Today, News from Indian Country, Bozeman Daily Chronicle, Helena Independent Record, Billings Gazette, Missoulian, Great Falls Tribune, West Yellowstone News, Livingston Enterprise, Montana Pioneer, Montana Standard, Flathead Beacon, Missoula Independent, Big Sky Weekly, Montana Public Radio, Pacifica Radio Stations, WBAI First Voices Indigenous Radio, KBZK-TV Bozeman, KXLF-TV Butte, ABC Montana, NBC Montana, CBS Montana, KGNU Colorado, Fox News Channel 8 Cleveland, Montana News Casper Star Tribune, Planet Jackson Hole, Jackson Hole News & Guide, Jackson Hole Weekly, Island Park News, Salt Lake Tribune, Powell Tribune, Ag Information Network, Idaho Statesman, Huffington Post, Word Press, New West, Yahoo! News, AlterNet, Mother Jones, Prairie Star, The Republic, Environmental News Service, Earth First! Journal, Mother Nature Network, CounterPunch, Animal People, Independent Media, multiple blogs and online news resources.

The language of the FOIA clearly indicates that Congress intended fees not to be a barrier to private individuals or public

interest organizations seeking access to government records. In addition, the legislative history of the FOIA fee waiver language indicates that Congress intended a liberal interpretation of the phrase "primarily benefiting the public." This suggests that all fees are to be waived whenever the release of information contributes to public debate on important policy issues. This has been affirmed by the US Court of Appeals for the District of Columbia, in *Better Government Association v. Department of State*, 780 F. 2d 86 (D.C. Cir. 1986). In that case, the court found that under the FOIA, Congress had explicitly recognized the need for non-profit organizations to have free access to government documents and those government agencies cannot impair this free access by charging duplication or search for FOIA information requests. *Id.* at 89.

I appreciate your help and prompt response. Thank you for your time.

Sincerely,

/s/
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
<http://www.buffalofieldcampaign.org>

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#); [Greiner, Steven J - APHIS](#)
Subject: New scan of Gonacon ACUC
Date: Tuesday, February 21, 2012 12:52:00 PM
Attachments: [ACUCBisonGonaConStudyfinal \(2\) with sigs.pdf](#)



I ended up rescanning it anyway☺

Let me know if it doesn't look right again.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

REGULATORY CONSIDERATIONS

Permits					
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <table style="width: 100%; border: none;"> <tr> <td style="border: none; width: 50%;">Permit(s) description</td> <td style="border: none; width: 25%;">Number</td> <td style="border: none; width: 25%;">Date</td> </tr> </table>	Permit(s) description	Number	Date
Permit(s) description	Number	Date			

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: _____Live animal work_____*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% shedding). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

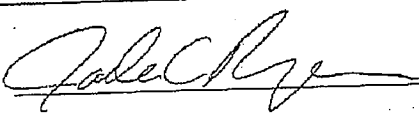
Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80


Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

PART ONE: SIGNATURE PAGE

Study Director:  Date: 5/16/11

Concur:
IACUC Chair  Date 5/16/11

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: Next week?
Date: Monday, December 30, 2013 3:52:00 PM

Hey there Becky and Ryan,

I hope you both are having a lovely holiday!

Could you fill me in on what the plan is for next week? Are we just working the GonaCon bison this time around? Do we need to bring anything besides our beautiful selves? Tubes? Swabs?

I will be in on the 2nd but out again from the 3rd to Sunday night so I'm hoping to get stuff together on the 2nd.

Talk soon! And happy New Year!

Pauline

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: [Jack C. Rhyan](#)
To: [Rebecca K. Frey](#); [Matt McCollum](#); [Pauline Nol](#)
Subject: "nuther draft of contraception protocol"
Date: Tuesday, February 01, 2011 2:57:00 PM
Attachments: [ImmunocontBisonProject_2-1-11.doc](#)

Please check it out and make any suggestions. this one has both seronegative calves and adults that pass quarantine being used for conservation. The devil is in the details so we will have to carefully design the process by which the critters are "used for conservation."

Jack

(See attached file: ImmunocontBisonProject_2-1-11.doc)

Proposed Project:**DRAFT**

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three

abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for B. abortus after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements and published in the UM&R will be used for bison conservation. (The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.) Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December til July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-021009,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Tuesday, December 23, 2014 2:32:55 PM
Attachments: [14-021009_DBL-BRUC_ALT_12-23-2014-03-17-54-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-021009

Date Received: 07/03/2014 10:39:55 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA, APHIS, VS
Corwin Springs, MT**Accession Number:** 14-021009**Animal Location**

Park County MT

Date Collected:**Date Received:** 07/03/2014**Submitter - 1961**

DR Patrick Ryan Clarke

Date Completed:**Collected By:** Frey Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Purpose:

General Diagnostic

Belgrade, MT 59714

FAX #: 406-866-5162

Referral Number:**This is not a billable case.**

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** Red09 **Animal ID:** Red09 **Brucella Case Number:** B14-0461 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3R22 **Animal ID:** 3R22 **Brucella Case Number:** B14-0462 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red26 **Animal ID:** Red26 **Brucella Case Number:** B14-0463 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Gr09 **Animal ID:** Gr09 **Brucella Case Number:** B14-0464 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Gr10 **Animal ID:** Gr10 **Brucella Case Number:** B14-0465 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Milk / Milk

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Placenta / Placenta

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3G08 **Animal ID:** 3G08 **Brucella Case Number:** B14-0466 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red13 **Animal ID:** Red13 **Brucella Case Number:** B14-0467 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Milk / Milk

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3G14 **Animal ID:** 3G14 **Brucella Case Number:** B14-0468 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red18 **Animal ID:** Red18 **Brucella Case Number:** B14-0469 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red20 **Animal ID:** Red20 **Brucella Case Number:** B14-0470 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red17 **Animal ID:** Red17 **Brucella Case Number:** B14-0471 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-021012,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Monday, January 05, 2015 3:32:08 PM
Attachments: [14-021012_DBL-BRUC_ALT_01-05-2015-05-31-11-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-021012

Date Received: 07/03/2014 10:44:05 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA, APHIS, VS-GonaCon Study
Corwin Springs, MT**Accession Number:****14-021012****Animal Location**

Park County MT

Date Collected:**Date Received:**

07/03/2014

Submitter - 1961

DR Patrick Ryan Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Date Completed:

01/05/2015

Collected By:

Frey Clarke

Purpose:

General Diagnostic

Referral Number:**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Red11 **Animal ID:** Red11 **Brucella Case Number:** B14-0472 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3R20 **Animal ID:** 3R20 **Brucella Case Number:** B14-0473 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B14-0474 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Gr03 **Animal ID:** Gr03 **Brucella Case Number:** B14-0475 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B14-0476 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red01 **Animal ID:** Red01 **Brucella Case Number:** B14-0477 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B14-0478 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B14-0479 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red28 **Animal ID:** Red28 **Brucella Case Number:** B14-0480 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red04 **Animal ID:** Red04 **Brucella Case Number:** B14-0481 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: Red14 **Animal ID:** Red14 **Brucella Case Number:** B14-0482 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B14-0483 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B14-0484 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 4G10 **Animal ID:** 4G10 **Brucella Case Number:** B14-0485 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab, Skin / Swab- Not Identified

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B14-0486 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, January 07, 2015 12:25:36 PM
Attachments: [14-029714_DBL-BRUC_ALT_01-07-2015-01-22-41-PM.pdf](#)

Submitter Name: Jack C Rhyan
Submitter Company: USDA, APHIS, VS
National Wildlife Research Center
Referral Number:
FAD Number:
Accession: 14-029714
Date Received: 09/16/2014 09:30:41 AM
Purpose: Developmental Research
Exam(s) Requested: BRUC
Submitter State: CO
Owner State: CO
Animal State: MT
Species: [Bison]



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Owner

Embryo Transfer Study
Ft. Collins, CO

Accession Number: 14-029714

Animal Location

Park County MT

Date Collected: 08/21/2014

Date Received: 09/16/2014

Submitter - 2649

DR Jack C. Rhyan
USDA, APHIS, VS
National Wildlife Research Center
4101 La Porte Ave
Fort Collins, CO 80521
FAX #: 970-266-6138
Phone #: 970-266-6140

Date Completed: 01/07/2015

Collected By: Jack Rhyan

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 49 **Animal ID:** 49 **Brucella Case Number:** B14-0564 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Placenta / Placenta

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Swab / Swab, Placenta

Brucella Isolation Result

Contaminated

Milk / Milk- Left Front

Brucella Isolation Result

No Isolation Made

Milk / Milk- Left Rear

Brucella Isolation Result

No Isolation Made

Milk / Milk- Right Front

Brucella Isolation Result

No Isolation Made

Milk / Milk- Right Rear

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Placenta and Placenta swab samples were very heavily contaminated. Both were overgrown with swarming bacteria, so the amount of Brucella could not be estimated.

Sample: 49 Calf **Animal ID:** 49 Calf **Brucella Case Number:** B14-0565 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Lymph Node Pool / Lymph Node- Not Identified

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Lymph Node / Lymph Node- Throacic Region

Brucella Isolation Result

Suspect Isolated

Lymph Node / Lymph Node- Abdominal Region

Brucella Isolation Result

Suspect Isolated

Spleen / Spleen

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Lung / Lung

Brucella Isolation Result

Suspect Isolated

Liver / Liver

Brucella Isolation Result

Suspect Isolated

Estimated amount of Brucella on inital isolation:**LN-NI: Confluent lawn of *Brucella*****Thoracic LN: Confluent lawn of *Brucella*****Abdominal LN: >300 colonies****Spleen: ~250 colonies****Lung: Confluent lawn of *Brucella*****Liver: ~250 colonies****Results authorized by:**Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**Help Us Help You**

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-032973,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, December 31, 2014 7:23:07 AM
Attachments: [14-032973_DBL-BRUC_ALT_12-31-2014-08-19-47-AM.pdf](#)

Submitter Name: Jack C Rhyan
Submitter Company: USDA, APHIS, VS
National Wildlife Research Center
Referral Number:
FAD Number:
Accession: 14-032973
Date Received: 10/09/2014 10:20:56 AM
Purpose: Developmental Research
Exam(s) Requested: BRUC
Submitter State: CO
Owner State:
Animal State: CO
Species: [Bison]



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Owner

Accession Number: 14-032973

Animal Location

Larimer County CO

Date Collected:

Date Received: 10/09/2014

Submitter - 2649

DR Jack C. Rhyan

USDA, APHIS, VS

National Wildlife Research Center

4101 La Porte Ave

Fort Collins, CO 80521

FAX #: 970-266-6138

Phone #: 970-266-6140

Date Completed: 12/30/2014

Collected By: Jack Rhyan

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 10 Animal ID: 10 Brucella Case Number: B14-0640 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 12 Animal ID: 12 Brucella Case Number: B14-0641 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: No Tag Animal ID: No Tag Brucella Case Number: B14-0642 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 58 Animal ID: 58 Brucella Case Number: B14-0643 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 59 Animal ID: 59 Brucella Case Number: B14-0644 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 292 Animal ID: 292 Brucella Case Number: B14-0645 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 66 Animal ID: 66 Brucella Case Number: B14-0646 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: 69 Animal ID: 69 Brucella Case Number: B14-0647 Specimen Type: Blood, Whole Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by:Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388**[Help Us Help You](#)**

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-041525,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Tuesday, December 30, 2014 2:01:31 PM
Attachments: [14-041525_DBL-BRUC_ALT_12-30-2014-02-58-15-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-041525

Date Received: 12/17/2014 10:43:48 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



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Owner

GonaCon Study - APHIS
Corwin Springs, MT

Accession Number: 14-041525

Animal Location

Park County MT

Date Collected: 08/13/2014

Date Received: 12/17/2014

Submitter - 1961

DR Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 12/30/2014

Collected By: R. Frey, etal

Purpose: General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red25 **Animal ID:** Red25 **Brucella Case Number:** B14-0700 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Milk / Milk

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Vaginal

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-041526,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Monday, January 05, 2015 3:32:21 PM
Attachments: [14-041526_DBL-BRUC_ALT_01-05-2015-05-31-13-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-041526

Date Received: 12/17/2014 11:01:24 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

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Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

USDA/APHIS/VS-GonaCon Study

Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

DR Patrick Ryan Clarke

USDA, APHIS, VS

187 E. Tobiano Tr.

Belgrade, MT 59714

FAX #: 406-866-5162

Phone #: 406-866-5162

Accession Number:**14-041526****Date Collected:**

07/15/2014

Date Received:

12/17/2014

Date Completed:

01/05/2015

Collected By:

R. Frey, et al

Purpose:

General Diagnostic

Referral Number:**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** Grn21 **Animal ID:** Grn21 **Brucella Case Number:** B14-0701 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Grn24 **Animal ID:** Grn24 **Brucella Case Number:** B14-0702 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: Grn25 **Animal ID:** Grn25 **Brucella Case Number:** B14-0703 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#14-041527,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, December 31, 2014 7:23:20 AM
Attachments: [14-041527_DBL-BRUC_ALT_12-31-2014-08-19-52-AM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 14-041527

Date Received: 12/17/2014 11:01:24 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



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Owner

USDA, APHIS GonaCon Study
Corwin Springs, MT

Animal Location

Park County MT

Submitter - 1961

DR Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Accession Number:

14-041527

Date Collected:

09/16/2014

Date Received:

12/17/2014

Date Completed:

12/30/2014

Collected By:

R. Frey, et al

Purpose:

General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Grn19 **Animal ID:** Grn19 **Brucella Case Number:** B14-0704 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: Grn31 **Animal ID:** Grn31 **Brucella Case Number:** B14-0705 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: Grn32 **Animal ID:** Grn32 **Brucella Case Number:** B14-0706 **Specimen Type:** Blood **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#15-005636,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Thursday, March 05, 2015 10:36:39 AM
Attachments: [15-005636_DBL-BRUC_ALT_03-05-2015-11-36-05-AM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-005636

Date Received: 02/19/2015 11:54:31 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



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Owner

USDA APHIS VS- GonaCon Study
Corwin Springs, MT

Accession Number: 15-005636

Animal Location

Park County MT

Date Collected:

Date Received: 02/19/2015

Submitter - 1961

DR Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 03/05/2015

Collected By: Clarke, Frey

Purpose: General Diagnostic

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 1-Red08 **Animal ID:** Red08 **Brucella Case Number:** B15-0094 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

Milk / Milk

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Feces / Feces

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Placenta / Placenta

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: NVSL Report - Accession#15-008065,Purpose:GEN_DIAG,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Monday, March 23, 2015 1:03:56 PM
Attachments: [15-008065_DBL-BRUC_ALT_03-23-2015-02-03-10-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-008065

Date Received: 03/11/2015 12:19:33 PM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



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Owner

USDA, APHIS, VS-GonaCon Study
Corwin Springs, MT

Accession Number: 15-008065

Animal Location

Park County MT

Date Collected:

Date Received: 03/11/2015

Submitter - 1961

DR Patrick Ryan Clarke

Date Completed: 03/23/2015

Collected By: Clarke, Frey

USDA, APHIS, VS

187 E. Tobiano Tr.

Purpose: General Diagnostic

Belgrade, MT 59714

FAX #: 406-866-5162

Referral Number:

This is not a billable case.

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: Red20 **Animal ID:** Red20 **Brucella Case Number:** B15-0100 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Uterine

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#15-015494,Purpose:GEN_DIAG,Exam Req:BRUC sent to Patrick.R.Clarke@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Friday, May 15, 2015 10:42:57 AM
Attachments: [15-015494_DBL-BRUC_FIN_05-15-2015-11-42-03-AM.pdf](#)

Submitter Name:

Submitter Company: MT Department of Livestock

Diagnostic Laboratory Division

Referral Number: 8-396-15

FAD Number:

Accession: 15-015494

Date Received: 05/13/2015 11:10:17 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#15-019452,Purpose:DEV_RES,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Thursday, July 16, 2015 11:36:28 AM
Attachments: [15-019452_DBL-BRUC_ALT_07-16-2015-12-06-36-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-019452

Date Received: 06/16/2015 11:49:42 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]



National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 Fax: 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

Owner

Bison Quarantine-G. Study
Corwin Springs, MT

Accession Number: 15-019452

Animal Location

Park County MT

Date Collected:

Date Received: 06/16/2015

Submitter - 1961

Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162

Date Completed: 07/16/2015

Collected By: R. Frey

Purpose: Development /
Research

Referral Number:

This is not a billable case.

NOTE: Condition of the sample(s) was adequate unless otherwise noted.

Sample: 3G03 **Animal ID:** 3G03 **Brucella Case Number:** B15-0238 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 3G17 **Animal ID:** 3G17 **Brucella Case Number:** B15-0239 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G08 **Animal ID:** 4G08 **Brucella Case Number:** B15-0240 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4G17 **Animal ID:** 4G17 **Brucella Case Number:** B15-0241 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R06 **Animal ID:** 4R06 **Brucella Case Number:** B15-0242 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R13 **Animal ID:** 4R13 **Brucella Case Number:** B15-0243 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 4R22 **Animal ID:** 4R22 **Brucella Case Number:** B15-0244 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R01 **Animal ID:** R01 **Brucella Case Number:** B15-0245 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R04 **Animal ID:** R04 **Brucella Case Number:** B15-0246 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R05 Animal ID: R05 Brucella Case Number: B15-0247 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R11 Animal ID: R11 Brucella Case Number: B15-0248 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R17 Animal ID: R17 Brucella Case Number: B15-0249 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R19 Animal ID: R19 Brucella Case Number: B15-0250 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R25 Animal ID: R25 Brucella Case Number: B15-0251 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R27 Animal ID: R27 Brucella Case Number: B15-0252 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R28 Animal ID: R28 Brucella Case Number: B15-0253 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R29 Animal ID: R29 Brucella Case Number: B15-0254 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: R31 Animal ID: R31 Brucella Case Number: B15-0255 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

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Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#15-020347,Purpose:GEN_DIAG,Exam Req:BRUC sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Thursday, July 30, 2015 11:08:03 AM
Attachments: [15-020347_DBL-BRUC_ALT_07-30-2015-11-56-27-AM.pdf](#)

Submitter Name: Patrick Ryan Clarke

Submitter Company: USDA, APHIS, VS

Referral Number:

FAD Number:

Accession: 15-020347

Date Received: 06/25/2015 10:24:26 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: MT

Owner State: MT

Animal State: MT

Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS
Corwin Springs, MT**Accession Number:** 15-020347**Animal Location**

Park County MT

Date Collected:**Date Received:** 06/25/2015**Submitter - 1961**

Dr. Patrick Ryan Clarke

Date Completed:**Collected By:** Clarke Frey

USDA, APHIS, VS

187 E. Tobiano Tr.

Purpose:

General Diagnostic

Belgrade, MT 59714

FAX #: 406-866-5162

Referral Number:**This is not a billable case.**

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** R22 **Animal ID:** R22 **Brucella Case Number:** B15-0286 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 5R22 **Animal ID:** 5R22 **Brucella Case Number:** B15-0287 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: G25 **Animal ID:** G25 **Brucella Case Number:** B15-0288 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 5G25 **Animal ID:** 5G25 **Brucella Case Number:** B15-0289 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Sample: R16 **Animal ID:** R16 **Brucella Case Number:** B15-0290 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Exudate / Exudate- Vaginal

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

Contaminated

Feces / Feces

Brucella Isolation Result

Contaminated

Sample: 5R16 **Animal ID:** 5R16 **Brucella Case Number:** B15-0291 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Blood / Blood

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:45:58 PM
Attachments: [16-025296_DBL-BRUC_ALT_10-05-2016-01-32-40-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke
Submitter Company: USDA, APHIS, VS
Referral Number: First Cohort
FAD Number:
Accession: 16-025296
Date Received: 08/05/2016 11:09:32 AM
Purpose: NVSL Interlab Diagnostic
Exam(s) Requested: BABORT
Submitter State: MT
Owner State: MT
Animal State: MT
Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon Study
Corwin Springs, MT**Accession Number:** 16-025296**Animal Location**

Park County MT

Date Collected:**Date Received:** 08/05/2016**Submitter - 1961**

Dr. Patrick Ryan Clarke

Date Completed: 10/05/2016

USDA, APHIS, VS

Collected By: Frey, Clarke

187 E. Tobiano Tr.

Purpose: NVSL Internal

Belgrade, MT 59714

Referral Number: First Cohort

FAX #: 406-866-5162

This is not a billable case.

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** 5G09 **Animal ID:** 5G09 **Brucella Case Number:** B16-0489 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 5R02 **Animal ID:** 5R02 **Brucella Case Number:** B16-0490 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B16-0491 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red28 Animal ID: Red28 Brucella Case Number: B16-0492 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red1 Animal ID: Red1 Brucella Case Number: B16-0493 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red5 Animal ID: Red5 Brucella Case Number: B16-0494 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red11 Animal ID: Red11 Brucella Case Number: B16-0495 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red14 Animal ID: Red14 Brucella Case Number: B16-0496 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red4 Animal ID: Red4 Brucella Case Number: B16-0497 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B16-0498 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B16-0499 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red25 **Animal ID:** Red25 **Brucella Case Number:** B16-0500 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B16-0501 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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(This new section will be updated periodically with tips for submitters.)

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#16-025296,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 12:45:58 PM
Attachments: [16-025296_DBL-BRUC_ALT_10-05-2016-01-32-40-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke
Submitter Company: USDA, APHIS, VS
Referral Number: First Cohort
FAD Number:
Accession: 16-025296
Date Received: 08/05/2016 11:09:32 AM
Purpose: NVSL Interlab Diagnostic
Exam(s) Requested: BABORT
Submitter State: MT
Owner State: MT
Animal State: MT
Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon Study
Corwin Springs, MT**Accession Number:** 16-025296**Animal Location**

Park County MT

Date Collected:**Date Received:** 08/05/2016**Submitter - 1961**Dr. Patrick Ryan Clarke
USDA, APHIS, VS
187 E. Tobiano Tr.
Belgrade, MT 59714
FAX #: 406-866-5162
Phone #: 406-866-5162**Date Completed:****Collected By:** Frey, Clarke**Purpose:** NVSL Internal**Referral Number:** First Cohort**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.****Sample:** 5G09 **Animal ID:** 5G09 **Brucella Case Number:** B16-0489 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: 5R02 **Animal ID:** 5R02 **Brucella Case Number:** B16-0490 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red29 **Animal ID:** Red29 **Brucella Case Number:** B16-0491 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red28 Animal ID: Red28 Brucella Case Number: B16-0492 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red1 Animal ID: Red1 Brucella Case Number: B16-0493 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red5 Animal ID: Red5 Brucella Case Number: B16-0494 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red11 Animal ID: Red11 Brucella Case Number: B16-0495 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red14 Animal ID: Red14 Brucella Case Number: B16-0496 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red4 Animal ID: Red4 Brucella Case Number: B16-0497 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red27 **Animal ID:** Red27 **Brucella Case Number:** B16-0498 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red19 **Animal ID:** Red19 **Brucella Case Number:** B16-0499 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red25 **Animal ID:** Red25 **Brucella Case Number:** B16-0500 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red31 **Animal ID:** Red31 **Brucella Case Number:** B16-0501 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
NVSL MB General Phone: 515-337-7388

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From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#16-025297,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 1:15:10 PM
Attachments: [16-025297_DBL-BRUC_ALT_10-05-2016-02-11-17-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke
Submitter Company: USDA, APHIS, VS
Referral Number: 2nd Cohort
FAD Number:
Accession: 16-025297
Date Received: 08/05/2016 11:16:21 AM
Purpose: NVSL Interlab Diagnostic
Exam(s) Requested: BABORT
Submitter State: MT
Owner State: MT
Animal State: MT
Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

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Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon Study
Corwin Springs, MT**Accession Number:** 16-025297**Animal Location**

Park County MT

Date Collected:**Date Received:** 08/05/2016**Submitter - 1961**

Dr. Patrick Ryan Clarke

Date Completed: 10/05/2016

USDA, APHIS, VS

Collected By: Frey, Clarke

187 E. Tobiano Tr.

Purpose: NVSL Internal

Belgrade, MT 59714

Referral Number: 2nd Cohort

FAX #: 406-866-5162

This is not a billable case.

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** Red35 **Animal ID:** Red35 **Brucella Case Number:** B16-0502 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 6R35 **Animal ID:** 6R35 **Brucella Case Number:** B16-0503 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Sample: Red32 Animal ID: Red32 Brucella Case Number: B16-0504 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red36 Animal ID: Red36 Brucella Case Number: B16-0505 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red39 Animal ID: Red39 Brucella Case Number: B16-0506 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red41 Animal ID: Red41 Brucella Case Number: B16-0507 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red42 Animal ID: Red42 Brucella Case Number: B16-0508 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red44 Animal ID: Red44 Brucella Case Number: B16-0509 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red45 Animal ID: Red45 Brucella Case Number: B16-0510 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red47 Animal ID: Red47 Brucella Case Number: B16-0511 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red48 Animal ID: Red48 Brucella Case Number: B16-0512 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red49 Animal ID: Red49 Brucella Case Number: B16-0513 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red51 Animal ID: Red51 Brucella Case Number: B16-0514 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red53 Animal ID: Red53 Brucella Case Number: B16-0515 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red54 Animal ID: Red54 Brucella Case Number: B16-0516 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red55 Animal ID: Red55 Brucella Case Number: B16-0517 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red56 Animal ID: Red56 Brucella Case Number: B16-0518 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red33 Animal ID: Red33 Brucella Case Number: B16-0519 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red37 Animal ID: Red37 Brucella Case Number: B16-0520 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red40 Animal ID: Red40 Brucella Case Number: B16-0521 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red50
Animal ID: Red50
Brucella Case Number: B16-0522
Specimen Type: Swab
Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red64
Animal ID: Red64
Brucella Case Number: B16-0523
Specimen Type: Swab
Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red67
Animal ID: Red67
Brucella Case Number: B16-0524
Specimen Type: Swab
Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red72
Animal ID: Red72
Brucella Case Number: B16-0525
Specimen Type: Swab
Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red74
Animal ID: Red74
Brucella Case Number: B16-0526
Specimen Type: Swab
Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by:

Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
 NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [APHIS-NVSL Case Coordinator - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: NVSL Report - Accession#16-025297,Purpose:NVSL_INTER,Exam Req:BABORT sent to Rebecca.K.Frey@aphis.usda.gov,Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov
Date: Wednesday, October 05, 2016 1:15:10 PM
Attachments: [16-025297_DBL-BRUC_ALT_10-05-2016-02-11-17-PM.pdf](#)

Submitter Name: Patrick Ryan Clarke
Submitter Company: USDA, APHIS, VS
Referral Number: 2nd Cohort
FAD Number:
Accession: 16-025297
Date Received: 08/05/2016 11:16:21 AM
Purpose: NVSL Interlab Diagnostic
Exam(s) Requested: BABORT
Submitter State: MT
Owner State: MT
Animal State: MT
Species: [Bison]

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

Phone: 515-337-7514 **Fax:** 515-337-7938

FEDERAL RELAY SERVICE (Voice/TTY/ASCII/Spanish) 1-800-877-8339

The USDA is an equal opportunity provider and employer.

FINAL REPORT

Laboratory Test Report

Sensitive But Unclassified/Sensitive Security Information - Disseminate on a Need-To-Know Basis Only

OwnerUSDA APHIS VS- GonaCon Study
Corwin Springs, MT**Accession Number:** 16-025297**Animal Location**

Park County MT

Date Collected:**Date Received:** 08/05/2016**Submitter - 1961**

Dr. Patrick Ryan Clarke

Date Completed: 10/05/2016

USDA, APHIS, VS

Collected By: Frey, Clarke

187 E. Tobiano Tr.

Purpose: NVSL Internal

Belgrade, MT 59714

Referral Number: 2nd Cohort

FAX #: 406-866-5162

This is not a billable case.

Phone #: 406-866-5162

NOTE: Condition of the sample(s) was adequate unless otherwise noted.**Sample:** Red35 **Animal ID:** Red35 **Brucella Case Number:** B16-0502 **Specimen Type:** Multiple - Specify in Sample **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Fluid / Fluid- Vaginal

Brucella Isolation Result

No Isolation Made

Milk / Milk

Brucella Isolation Result

No Isolation Made

Feces / Feces

Brucella Isolation Result

No Isolation Made

Sample: 6R35 **Animal ID:** 6R35 **Brucella Case Number:** B16-0503 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Conjunctival

Brucella Isolation Result

No Isolation Made

Sample: Red32 Animal ID: Red32 Brucella Case Number: B16-0504 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red36 Animal ID: Red36 Brucella Case Number: B16-0505 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red39 Animal ID: Red39 Brucella Case Number: B16-0506 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red41 Animal ID: Red41 Brucella Case Number: B16-0507 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red42 Animal ID: Red42 Brucella Case Number: B16-0508 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red44 Animal ID: Red44 Brucella Case Number: B16-0509 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red45 **Animal ID:** Red45 **Brucella Case Number:** B16-0510 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red47 **Animal ID:** Red47 **Brucella Case Number:** B16-0511 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red48 **Animal ID:** Red48 **Brucella Case Number:** B16-0512 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red49 **Animal ID:** Red49 **Brucella Case Number:** B16-0513 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red51 **Animal ID:** Red51 **Brucella Case Number:** B16-0514 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red53 **Animal ID:** Red53 **Brucella Case Number:** B16-0515 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red54 Animal ID: Red54 Brucella Case Number: B16-0516 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red55 Animal ID: Red55 Brucella Case Number: B16-0517 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red56 Animal ID: Red56 Brucella Case Number: B16-0518 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red33 Animal ID: Red33 Brucella Case Number: B16-0519 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red37 Animal ID: Red37 Brucella Case Number: B16-0520 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red40 Animal ID: Red40 Brucella Case Number: B16-0521 Specimen Type: Swab Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red50 **Animal ID:** Red50 **Brucella Case Number:** B16-0522 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red64 **Animal ID:** Red64 **Brucella Case Number:** B16-0523 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red67 **Animal ID:** Red67 **Brucella Case Number:** B16-0524 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red72 **Animal ID:** Red72 **Brucella Case Number:** B16-0525 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Sample: Red74 **Animal ID:** Red74 **Brucella Case Number:** B16-0526 **Specimen Type:** Swab **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

Swab / Swab- Vaginal

Brucella Isolation Result

No Isolation Made

Results authorized by: Dr. Suelee Robbe-Austerman, Section Head, Mycobacteria and Brucella Section
 NVSL MB General Phone: 515-337-7388

Help Us Help You

(This new section will be updated periodically with tips for submitters.)

Quality samples yield the most accurate results. Please call if you have questions.

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: Pregnant GnRH cows
Date: Thursday, January 17, 2013 1:25:30 PM

Were moved this morning.

Matt

From: [Jack C Rhyan](#)
To: [Rebecca K Frey](#)
Cc: [Pauline Nol](#); [Matt McCollum](#); [Brian J McCluskey](#); [Patrick R Clarke](#)
Subject: protocol updated
Date: Tuesday, April 05, 2011 1:51:00 PM
Attachments: [ImmunocontBisonProject_4-5-11.doc](#)

Becky,

Here is the updated version where we have tried to accommodate many of the Park's ideas with the hope that they will enter into this with us. I included Rick and Jenny on it. If they balk, strike them. Also please go through it and correct errors, etc. It still needs some fleshing out but hopefully will serve our purpose for now.

Thanks much, Becky.

Jack

(See attached file: ImmunocontBisonProject_4-5-11.doc)

Proposed Project:**DRAFT**

Title: Evaluation of sterilization by use of and GonaCon™, an immunocontraceptive vaccine, and ovariectomy as means of decreasing the potential for transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

USDOI, NPS: Rick Wallen, Jenny Powers

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary to occasionally long term or permanent infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg GnRH compound. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing shedding and transmission of *B. abortus* which leads to persistence of the disease in populations.

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.
2. Evaluate the effect of sterilization produced by ovariectomy of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.

3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous or sterilization by ovariectomy has on *B. abortus* colonization in naturally-infected female bison; determine if pregnancies following infertility would result in non-infectious parturition.
4. Determine the effect of immune system stimulation via vaccination with Adjuvac (Gonacon's adjuvant) on brucella titers and shedding

Research Plan:

A total of at least 80 and not more than 100 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 40 to 60 seronegatives and 40 seropositives) and 8 seronegative bulls captured in late winter/spring 2011 and, if needed 2012 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility up to one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 80 female bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into four pastures, each containing 10 seropositives and 10 to 15 seronegative “sentinels” and 2 bulls. Seropositive bison in the four pastures will be treated as follows:

Pasture A (GonaCon treatment) will contain 10 seropositive females vaccinated with GonaCon™ vaccine (containing 3000µg), 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

Pasture B (Untreated control) will contain 10 seropositive female non-vaccinates, 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

Pasture C (Ovariectomized “gold standard control”) will contain 10 seropositive ovariectomized female bison, 10 to 15 seronegative female bison and 2 seronegative bulls.

Pasture D (Adjuvant-only treated controls) will contain 10 seropositive female bison treated with Adjuvac™, 10 to 15 seronegative female bison and 2 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013 – 2017). Bulls will be separated from the cows after breeding season, from December until July. During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture. All bison will be tested by serology and culture in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate.

Spring 2012 – Apply treatments: 1. GnRH, 2. Adjuvac, 3. ovariectomize . Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2017 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Conclusion of study – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. Determine the effectiveness of the permanent sterility produced by ovariectomy and temporary sterility produced by use of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds
2. Determine the effect of prolonged anestrus produced by GonaCon™ and ovariectomy on the survival of *B. abortus* in infected bison. Determine if contracepted female bison become shedders of *B. abortus* after resumption of reproduction.
3. Determine the effect of adjuvant alone on shedding and transmission in seropositive dams
4. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Appendix: Sample size calculation:

Pasture A Seroconversion	Sample size per group				
0.5	407	103	45	24	14
0.4	107	49	28	17	11
0.3	49	29	19	13	9
0.2	28	19	13	10	8
0.1	17	13	10	8	6
0.01	11	9	8	6	5
	0.6	0.7	0.8	0.9	0.99
	Pasture B Seroconversion				

It is anticipated in a fenced enclosure that a single abortion or shedding event will result in the infection of a majority of the sentinels, and no abortion or shedding event will result in no infection of the sentinels. Therefore a sentinel sample size of 10 to 15 should be adequate. Based on previous studies, at least 30% of young seropositive females are expected to abort in the course of 5 years.

From: [Pauline Nol](#)
To: [Rebecca K Frey](#)
Subject: protocol with RW edits
Date: Wednesday, March 09, 2011 8:40:00 AM
Attachments: [Rhyan Immunocontraception Study Plan_rlw review.doc](#)
[ATTCMHS6.doc](#)
[USAHA Committee on Wildlife Diseases.doc](#)

----- Forwarded by Pauline Nol/CO/APHIS/USDA on 03/09/2011 08:39 AM -----

Jack C
Rhyan/CO/APHIS/USDA
05/25/2010 11:47 AM

To Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA
cc
Subject Fw: USAHA Brucellosis committee

interesting

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 05/25/2010 11:47 AM -----

Andy Schwartz
<Andy.Schwartz@tahc.state.tx.us>
05/25/2010 09:36 AM

To Jim Logan <jjlogan1@state.wy.us>, Keith
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SubjectRE: USAHA Brucellosis committee

Jim,
 We continue to encounter diagnostic challenges due to B. suis in cattle. We've

cultured the organism from milk and/or tissue in 29 animals in the past 3 years, and are compelled to do an extensive workup to rule out B. abortus in many more animals with reactor level titers. I feel this is a growing issue, and any state with feral swine either has or will soon have to deal with it. If the committee is interested, perhaps someone could suggest a speaker or speakers to address some of the issues at hand, such as any potential serological assay to differentiate B. suis, and additional research to determine if cattle really are dead end hosts for B. suis.

Respectfully submitted,
Andy

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-----Original Message-----

From: Jim Logan [<mailto:jlogan1@state.wy.us>]
Sent: Friday, May 21, 2010 12:52 PM
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Cc: Betsy Milek; brichey@usaha.org; kelly@usaha.org
Subject: USAHA Brucellosis committee

I would like to start assembling the agenda for the fall Brucellosis committee meeting.

I had earlier sent out a request for suggestions for scientific papers and abstracts to be presented at either the meeting or USAHA general session and have only received one response from that request. Thank you for that, Dr Wolfram.

If there are others, please let me know asap so I can inform Ben Richey. Please send me your suggestions for topics and speakers. I would like to have the agenda set by July 1 if possible to give us plenty of time to contact speakers, etc.

Please also cc Drs Bill Barton and Tony Frasier co- vice chairs and also my secretary, Betsy Milek. Their e mail addresses are:

Betsy: bmilek@state.wy.us

Dr Barton: bill.barton@agri.idaho.gov

Dr Frasier: stvet@agi.alabama.gov

Thanks,
Jim

Dr. Jim Logan, DVM
State Veterinarian
Wyoming Livestock Board
State of Wyoming

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----- Forwarded by Pauline Nol/CO/APHIS/USDA on 03/09/2011 08:39 AM -----

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SubjectUSAHA Committee on Wildlife Diseases

Greetings to all our committee members and speakers!

The Committee on Wildlife Diseases will meet on Tuesday morning, November 16, from 8 AM until 12 Noon in Salon B of the Minneapolis Hilton. The agenda for the meeting is attached.

As you can see, the agenda is completely full and it will be necessary for all speakers to stay within their allotted times in order for us to adjourn on schedule. All speakers must have their PowerPoint presentations to Colin Gillin (colin.m.gillin@state.or.us) before the start of the meeting. Speakers also must have a brief written summary of their presentations to Colin and me (schmitts@michigan.gov) prior to the meeting so they can be included in the committee report and published in the USAHA Proceedings.

If any of you have resolutions you would like to bring up for committee consideration, please send them to us prior to the meeting so they can be distributed to all of our members. We look forward to seeing you in Minneapolis.

Colin and Steve

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(See attached file: USAHA Committee on Wildlife Diseases.doc)

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Martin" <MZaluski@mt.gov>, (b) (6)
(b) (6) @rea-alp.com>

cc

Subject: Agenda for USAHA Committee on Wildlife
Disease

Greetings to all our committee members,

The Committee on Wildlife Diseases will meet on Tuesday morning, November 16, from 8 AM until 12 Noon in Salon B of the Minneapolis Hilton. The final agenda is attached. At this time, there are no resolutions, but I am aware of a couple that might be brought forward and will distribute them when I receive them.

We look forward to seeing you in a couple of weeks.

Colin and Steve

Stephen M. Schmitt, D.V.M.
Michigan Department of Natural Resources & Environment
Wildlife Disease Laboratory
4125 Beaumont Road, Room 250
Lansing, MI 48910-8106
Phone: 517-336-5030
FAX: 517-337-4920
email: schmitts@michigan.gov

(See attached file: USAHA Committee on Wildlife Diseases.doc)

----- Forwarded by Pauline Nol/CO/APHIS/USDA on 03/09/2011 08:39 AM -----

Jenny_Powers@nps.gov

12/20/2010 03:23 PM

(b) (6) @gmail.com, (b) (6) berry.edu,
JMills@cdc.gov, Joshua Dein <fjdein@usgs.gov>,
(b) (6) @u.washington.edu"
(b) (6) @u.washington.edu>, "Rose, Karrie"
<krose@zoo.nsw.gov.au>, (b) (6)
(b) (6) @uwoyo.edu>, "Powers, Jenny"
<jenny_powers@nps.gov>, Jane Harms
<naomi_harms@usask.ca>,
(b) (6) @tufts.edu"
(b) (6) @tufts.edu>, "rick_wallen@nps.gov"
<rick_wallen@nps.gov>,
colin m.gilllin@state.or.us, Erik.Agren@sva.se,
t kuiken@erasmusmc.nl,
(b) (6) @gmail.com, naomi.harms@usask.ca,
(b) (6) @gmail.com, (b) (6) @tufts.edu,
kristin.mansfield@dfw.wa.gov,
(b) (6) @ucdavis.edu,
(b) (6) @gmail.com,
charles_van_riper@usgs.gov,
pauline nol@aphis.usda.gov, (b) (6) @gmail.com,
dolores@sva.se, lynn.h.creekmore@aphis.usda.gov
cc: (b) (6) @rogers.com>

Subject: January Newsletter

Hello WDA Council members and information committee members,

I am in search of content for the January edition of the WDA newsletter. I have a feature article by Dave, Buffy, and Jim, on the need for involvement in the journal and publishing process. I am looking for section news, job announcements, and member activities.

Please send submissions by January 1st for publication on January 15th.

Thanks,
Jenny

Jenny Powers, DVM
Wildlife Veterinarian
National Park Service
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525

Phone: (970) 267-2122
Cell (b) (6)
Fax: (970) 225-3585

----- Forwarded by Pauline Nol/CO/APHIS/USDA on 03/09/2011 08:39 AM -----

Jack C ToPauline Nol/CO/APHIS/USDA@USDA
Rhyan/CO/APHIS/USDA cc
02/28/2011 11:28 AM SubjectFw: study proposal and capture recipe

FYI

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 02/28/2011 11:28 AM -----

Rick_Wallen@nps.gov ToJack.C.Rhyan@aphis.usda.gov
cc
02/28/2011 11:07 AM Subjectre: study proposal and capture recipe

(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

I included some thoughts and suggested edits in the attached study plan. I suggested that our Veterinary Staff in Ft. Collins be co investigators but have not received any replies from them. I will follow up with a phone call to check in with Margaret and Jenny.

We ended the field season using an Etorphine recipe with 1.3 ml high potency etorphine +0.2 ml (20 mg) xylazine (Reversed with 10 ml Naltrexone). We provide extra naltrex to prevent renarc problems in the presence of predators. If you calculate it out you can probably reverse with about 7 ml. See ZooPharm website.

RW(See attached file: Rhyan Immunocontraception Study Plan_rlw review.doc)

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria ~~occurrence of following~~ pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission the shedding~~ of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals.

The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the

UM&R will be used-made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Commented [r1]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r2]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r3]: Why not get the culture done as soon as possible?

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in ~~reducing transmission of *B. abortus* in bison herds will be determined~~ preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector.
 - 1- Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting?
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy, whether they would have an infectious live birth, or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

Formatted: Indent: Left: 1", No bullets or numbering

USAHA Committee on Wildlife Diseases
November 16, 2010, 8:00AM – 12:00 PM
Salon B
Minneapolis Hilton
Minneapolis, Minnesota

Agenda

Dr. Stephen M. Schmitt, Chair and Dr. Colin M. Gillin, Co-Chair

Bighorn Sheep

8:00-8:05	Introductory Comments	Steve Schmitt, Colin Gillin
8:05-8:25	Report of the Wild/Domestic Sheep Working Group	Walt Cook
8:25-8:50	Pneumonia in Bighorn Sheep	Sri Subramaniam

Cervids and Bison

8:50-9:05	Bovine Tuberculosis in Minnesota Wildlife	Erika Butler
9:05-9:20	CWD National Program	Pat Klein
9:20-9:40	Brucellosis Transmission Dynamics in the Northern GYA	Brant Schumaker
9:40-9:55	Brucellosis Challenges in GYA	Marty Zaluski
9:55-10:10	Brucellosis in Wildlife in the GYA	Mark Drew
10:10-10:25	Hemorrhagic Disease	Mark Ruder

Wolves, Bats, Wild Birds and Prairie Dogs

10:25-10:40	Echinococcus in Wolves	Mark Drew
10:40-11:00	White-Nose Syndrome in Bats	David Blehert
11:00-11:15	Avian Influenza Research	Justin Brown
11:15-11:30	Oral Plaque Vaccine	Tonie Rocke

Committee Business

11:30-12:00	Resolutions and Other Committee Business	Steve Schmitt, Colin Gillin
-------------	--	-----------------------------

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: PS...you have her 1st calf. 3R88
Date: Tuesday, January 27, 2015 3:01:10 PM

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Eckery, Douglas C - APHIS](#)
Subject: PSPB results on GonaCon Study
Date: Monday, December 09, 2013 11:07:05 AM
Attachments: [LOG 120513009 Rhyan - USDA National Wildlife Research Center 120513009 1386367642101.html](#)

From: BioTracking Testing Lab [mailto:testinglab@biotracking.com]

Sent: Friday, December 06, 2013 3:47 PM

To: Rhyan, Jack C - APHIS

Subject: BioPRYN Report for Bison & Buffalo

Dear BioPRYN Customer,

Here is the report on the samples we received from you. It's attached as an HTML file, and you should be able to directly open the attachment by double-clicking on it.

For up-to-date schedule and pricing information please visit our website at www.biotracking.com and click on Lab Services. Holiday closures and schedules are posted under Lab Services as well. We recommend checking the schedule prior to shipping samples to ensure you will receive results when expected. If you have any questions regarding the schedule, please do not hesitate to call and ask.

As always, we stand by our products and services, so please contact us here with any questions or comments you may have.

Thank you,

Amber Merk

Director of Laboratory Service & Sales

BioTracking LLC

1150 Alturas Dr. Ste. 105

Moscow, ID 83843

Office: 208.882.9736

Cell: (b) (6)

amerk@biotracking.com or testinglab@biotracking.com

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BioPRYN PSPB Report

Date Received	Log In #
12/5/2013	120513009

Submitted By	Report To
USDA National Wildlife Research Center 4101 LaPorte Avenue Fort Collins, CO 80521	Jack Rhyan

jack.c.rhyan@aphis.usda.gov

REPORT NOTES:
Mail Report

Report Date	Assay/Animal	Number of Samples
12/06/2013	Bison - 19 sample(s)	19

Open	Low Recheck	Cutoff	High Recheck	Pregnant
OD < 0.135	OD = 0.135 to 0.15	0.15	OD = 0.15 to 0.21	OD > 0.21

Tube Number	Animal ID	Response in Test, OD	PSPB Range	Days Post Breeding
1	Yellow 1	0.3697	Pregnant	40
2	Yellow 2	0.0639	Open	40
3	Yellow 3	0.0644	Open	40
4	Yellow 4	0.064	Open	40
5	Yellow 5	0.0583	Open	40
6	Yellow 6	0.0646	Open	40
7	Yellow 7	0.0692	Open	40
8	Yellow 8	0.0853	Open	40
9	Yellow 9	0.0798	Open	40
10	Yellow 10	0.07	Open	40
11	Green 11	0.0781	Open	40
12	Green 12	0.6185	Pregnant	40
13	Green 14	0.2884	Pregnant	40
14	Green 15	0.3439	Pregnant	40
15	Green 16	0.0772	Open	40
16	Green 17	0.5002	Pregnant	40
17	Green 18	0.4087	Pregnant	40
18	Green 19	0.4555	Pregnant	40
19	Green 20	0.0699	Open	40

BioPRYN measures the presence of Pregnancy-Specific Protein B (PSPB) in serum and the attached results are provided for your interpretation. If a sample's OD falls in the Open range, 99.9% of animals are not pregnant in

003045

confirmatory testing; alternatively, if the OD falls in the Pregnant range, 93 - 95% of animals are pregnant in confirmatory testing. Visit the website listed on this report for more detailed information about the BioPRYN test.

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From: [Nol, Pauline - APHIS](#)
To: [Greiner, Steven J - APHIS](#); [Bens, Catherine M - APHIS](#); [Greiner, Laura B - APHIS](#)
Subject: QA 1858
Date: Thursday, February 16, 2012 10:52:00 AM

Good morning!

I was wondering if you knew what the status was on this protocol: QA 1858 **Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#)
Subject: QA number
Date: Friday, December 30, 2011 10:10:00 AM

Hi Laura,

I either need a new QA# for the study: **Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

or I need to be reminded of it☺

Thanks and happy New Year!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6138

From: [Greiner, Laura B - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: QA-1858 approved
Date: Thursday, February 23, 2012 1:47:02 PM
Attachments: [QA-1958 Rhyan Evaluation of GonaCon vaccine as a means of decreasing shedding of Brucella abortus in bison.pdf](#)

Jack and Pauline,

Attached is the approved protocol. A hard copy is in the mail to Pauline. Contact me if you have any questions.

Laura Greiner

Quality Assurance Specialist | 970-266-6022 | laura.b.greiner@aphis.usda.gov
National Wildlife Research Center | 4101 LaPorte Avenue | Fort Collins, CO 80521

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	Development of injectable and oral contraceptive technologies and their assessment for wildlife population and disease management

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: Jack C. Myers Date: 2/17/12

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:

Date _____ ☐ Res. Scientist ☐ Proj. Leader

☒ Research Scientist☐ Project Leader

☒ Visiting Scientist: NWRC Representative/Contact: LOWEN MILLER

☐ Student: NWRC Representative/Contact: _____

Concur: _____
NWRC Research Project Leader Jacob Ryan Date 2/17/12

Review and Processing: L. Aheiser Date 2/21/12

Concur: NWRC Assistant Director Mark E. Robin Date 2/22/12

Approved: _____ Date 2/22/12

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: <u>June 2, 2011</u>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement . Material Transfer agreements will be developed prior to material transfer
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?

If yes, attach Analytical Chemistry Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

4. Schedule

Proposed Experimental Start Date: April 15, 2012
 Proposed Experimental Termination Date: October 1, 2017
 Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

- | | |
|------|---|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer (<i>Odocoileus virginianus</i>): Pivotal target animal safety study |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (<i>Dama dama</i>) at Point Reyes National Seashore, California |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (<i>Cervus elaphus</i>) at Rocky Mountain National Park, Colorado |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (<i>Equus caballus</i>) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer |

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Null Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for histopathologic, bacteriologic, and molecular analysis. These will include: lymph nodes (bronchial, hepatic, internal iliac, popliteal, mandibular, parotid, prescapular, medial retropharyngeal, and supramammary), mammary gland tissue, spleen, lung, liver ovaries, uterus, cervix, adrenal glands, pituitary gland, and vaccination sites. Vaccinated cows will be euthanized in the chute via captive bolt and exsanguination or high-powered rifle. Alternatively they will be sedated, followed up with captive bolt and exsanguination. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d. Animal calving observation records
 - e. Pregnancy assessment records
- D. Final Report

13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400
E. Animal Care Cost	\$0	\$0	\$0					
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer

with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E. 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Yoder, C. A. and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title) _____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

A). Animal Information:

Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

B1) Rationale for involving animals:

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

B2) Rationale for numbers to be used: If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

B3) Rationale for appropriateness of the species to be used: Bison are the target species.

C) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

D) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

E) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

F) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

G) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

J) Dietary contaminant exposure NA

K) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

L) Animal pain or distress

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ____ Patrick Ryan Clarke _____

Date of Consultation: ____ 13 May 2011 _____

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

M) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_ See attached__

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyan has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

From: [Mora, Darcy - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Wehtje, Morgan E - APHIS](#)
Cc: [Eckery, Douglas C - APHIS](#)
Subject: QA-1858 MT Bison GonaCon Study Questions
Date: Wednesday, November 16, 2016 2:51:43 PM

Hi All,

We analyzed several hundred bison serum samples this week to determine anti-GnRH antibody levels. I am currently trying to make sense of this massive amount of data and wondering if one of you might be available at some point this afternoon to answer a couple of quick questions about the study design. I will be out of the office beginning tomorrow, returning on 11/28, but I would love to share this data before heading out if possible. Please let me know if anyone has a few minutes available today!

Thank you,

Darcy Orahood Mora
Biologist
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone (970) 266-6061

From: [Mora, Darcy - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Wehtje, Morgan E - APHIS](#)
Cc: [Eckery, Douglas C - APHIS](#)
Subject: QA-1858 MT Bison GonaCon Study Questions
Date: Wednesday, November 16, 2016 2:51:43 PM

Hi All,

We analyzed several hundred bison serum samples this week to determine anti-GnRH antibody levels. I am currently trying to make sense of this massive amount of data and wondering if one of you might be available at some point this afternoon to answer a couple of quick questions about the study design. I will be out of the office beginning tomorrow, returning on 11/28, but I would love to share this data before heading out if possible. Please let me know if anyone has a few minutes available today!

Thank you,

Darcy Orahood Mora
Biologist
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone (970) 266-6061

From: [Bens, Catherine M - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Cc: [Greiner, Steven J - APHIS](#); [Greiner, Laura B - APHIS](#)
Subject: QAU review protocol QA-1858
Date: Thursday, February 16, 2012 4:39:12 PM

Hello Jack/Pauline,
The QA Unit has reviewed your recently submitted protocol and has minor comments below requiring your attention.
Please contact me with any questions or comments.

Cat

QA-1858: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

NWRC Protocol Classification: 4

GLP Classification: This study is described as a non-regulated with regard to current QA and GLP regulations. It will not require an inspection by the QAU.

COMMENTS:

PART ONE

Signature page: Please obtained new signatures for Jack once comments have been addressed.

PART TWO

Material Transfer Agreement: Biological materials submitted to outside labs, especially MVDL, will need a Material Transfer Agreement. Please check yes and attach a MTA or indicate that a MTA will be developed as part of study conduct.

PART FOUR:

Section 3. Sponsor: Please complete the contract no information. If there is no contract number indicate "NA" or equivalent.

Section 4, Schedule: As Pauline and I discussed, please change the experimental start date to May 2012 as the date test material is applied to test system. Pre-study quarantine are not considered part of this study to my knowledge.

Section 11. SOPs: Generally SOPs included are study specific rather than general NWRC SOPs. Consider removing AD001 and AD002 from this list especially since this study will not be monitored by the QA Unit.

Section 15. Staff Qualification: The section on Dr. Clarke appears to need some editing.

NEPA: In the section marked 'This study does not qualify for a Categorical Exclusion. Please provide information on the EA that is in development.

Catherine Bens

Quality Assurance Manager

USDA/APHIS/WS National Wildlife Research Center

Fort Collins, CO 80521

Phone (970) 266.6053

Cell (b) (6)

Fax (970) 266.6010

Catherine.m.bens@aphis.usda.gov

From: Frey, Rebecca K - APHIS
Sent: Friday, November 24, 2017 4:13 PM
To: Nol, Pauline - APHIS
Cc: Wehtje, Morgan E - APHIS
Subject: Quarantine bison serum for Steve

HI! Happy day after turkey day!

I know you prolly don't want to hear this but I came across more QT serum while looking for all of the GonaCon serum. I wasn't sure if it was duplicate or not so I went through it. I did wind up finding some serum from animals that were missing the 6/19/06 or 10/05/06 sample. The sample is from 8/9/06. Do we want to ship it in to fill in some gaps in the data? If we have a 6/19 or a 10/5 but not both is that good enough? I can't remember what we were thinking then!!
Thanks for the help....hope your memory is better than mine.
Becky

Rebecca Frey
Wildlife Biologist
USDA APHIS VS
Montana
406-697-9991

From: Anderson, Lori F - APHIS
 Sent: Monday, October 23, 2017 8:01 AM
 To: Rhyhan, Jack C - APHIS
 Cc: Robbe Austerman, Suelee - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Sprecher, Megan L - APHIS; Anderson, Lori F - APHIS; Schmitt, Beverly J - APHIS; Ostlund, Eileen N - APHIS; Anderson, Lori F - APHIS
 Subject: RE: ACTION: NEW FREEDOM OF INFORMATION REQUEST: 2018-APHIS-00286

Jack:
 It looks like this was due back last Friday. How long of an extension do I need to request from Mildred?

Lori F. Anderson
 Chief of Staff
 National Veterinary Services Laboratories
 USDA, APHIS, VS, STAS
 P.O. Box 844
 1920 Dayton Avenue
 Ames, IA 50010
 Desk: (515) 337-7405
 Mobile: (b) (6)

From: Sprecher, Megan L - APHIS
 Sent: Friday, October 20, 2017 4:01 PM
 To: Rhyhan, Jack C - APHIS <Jack.C.Rhyhan@aphis.usda.gov>; Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Anderson, Lori F - APHIS <Lori.F.Anderson@aphis.usda.gov>
 Cc: Wright, Brenda K - APHIS <Brenda.K.Wright@aphis.usda.gov>; Swanson, Marjorie J - APHIS <Marjorie.J.Swanson@aphis.usda.gov>
 Subject: RE: ACTION: NEW FREEDOM OF INFORMATION REQUEST: 2018-APHIS-00286

Hi Jack - I've looped in Lori Anderson, the NVSL POC for FOIAs. Will you please send your response to Lori Anderson vs. Brenda? Because it's related to NVSL, we'll need to be sure that Dr. Schmitt clears the response before it goes to FOIA.

Megan L. Sprecher, MPA
 Chief of Staff

Science, Technology, and Analysis Services
 1920 Dayton Ave.
 Ames, IA 50010

(o) 515 337 6729 | (c) (b) (6)
 megan.l.sprecher@aphis.usda.gov

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From: Rhyhan, Jack C - APHIS
 Sent: Friday, October 20, 2017 11:26 AM
 To: Swanson, Marjorie J - APHIS <Marjorie.J.Swanson@aphis.usda.gov>; Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Herriott, Donald E - APHIS <Don.E.Herriott@aphis.usda.gov>; Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Clarke, Patrick R. - APHIS <Patrick.R.Clarke@aphis.usda.gov>
 Cc: Sprecher, Megan L - APHIS <Megan.L.Sprecher@aphis.usda.gov>; Wright, Brenda K - APHIS <Brenda.K.Wright@aphis.usda.gov>
 Subject: RE: ACTION: NEW FREEDOM OF INFORMATION REQUEST: 2018-APHIS-00286

Question: We are preparing a publication that will detail the abortions, live calves, etc. Some of our emails about the project discuss that. Are we bound to release this info to BFC before publication?
 Jack

From: Swanson, Marjorie J - APHIS
 Sent: Friday, October 20, 2017 9:46 AM
 To: Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>; Rhyhan, Jack C - APHIS <Jack.C.Rhyhan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum,

003071

Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>
 Cc: Sprecher, Megan L - APHIS <Megan.L.Sprecher@aphis.usda.gov>; Wright, Brenda K - APHIS <Brenda.K.Wright@aphis.usda.gov>
 Subject: ACTION: NEW FREEDOM OF INFORMATION REQUEST: 2018-APHIS-00286
 Importance: High

Good morning -- Please see attachment 1 for an explanation of what we need to provide for this FOIA. It request relates to the evaluation of GonaCon.

Tho Don Herriott, SPRS, is named in the letter, tho he is referring this back to WildIT for action.

Please read through the request and provide documents to Brenda Wright (CEAH). If something is sensitive and you don't think it should be released, mark it as such and we will flag it for the FOIA staff. If you have it, provide it as soon as you can. If you don't have anything, drop Brenda an e-mail saying so.

Any questions, please contact Brenda or me. Thank you! Marj

Marj Swanson
 Administrative Officer
 Center for Epidemiology & Animal Health
 2150 Centre Ave, Building B, MS2E3
 Fort Collins, CO 80526
 Office Phone: (970) 494-7203
 Cell Phone: (b) (6) or (b) (6)

From: Wright, Brenda K - APHIS
 Sent: Friday, October 20, 2017 8:50 AM
 To: Swanson, Marjorie J - APHIS <Marjorie.J.Swanson@aphis.usda.gov>
 Cc: Bundy, Mildred O - APHIS <Mildred.Bundy@aphis.usda.gov>; Wright, Brenda K - APHIS <Brenda.K.Wright@aphis.usda.gov>
 Subject: FW: NEW FOIA: 2018-APHIS-00286

Hi Marj, This is the FOIA that we were discussing about. Thanks for straightening this one out.

Brenda K. Wright
 Staff Assistant
 Center for Epidemiology & Animal Health
 Science, Technology, & Analysis Services
 USDA APHIS Veterinary Services
 Phone: 970-494-7202
 Email: Brenda.k.wright@aphis.usda.gov
 The greatest achievements are those that benefit others.

From: Fonville, Lisa F - APHIS
 Sent: Tuesday, October 17, 2017 9:32 AM
 To: Wright, Brenda K - APHIS <Brenda.K.Wright@aphis.usda.gov>
 Cc: Carnahan, Julia S - APHIS <Julia.S.Carnahan@aphis.usda.gov>; Bundy, Mildred O - APHIS <Mildred.Bundy@aphis.usda.gov>
 Subject: NEW FOIA: 2018-APHIS-00286

Hi Brenda,

Mildred Bundy is travelling today and asked that I direct the attached NEW FOIA REQUEST to you for Action.

From: Fonville, Lisa F - APHIS
 Sent: Tuesday, October 17, 2017 7:54 AM
 To: Bundy, Mildred O - APHIS <Mildred.Bundy@aphis.usda.gov>; Carnahan, Julia S - APHIS <Julia.S.Carnahan@aphis.usda.gov>
 Cc: APHIS-VS SPRS ADA Assistants <VS.SPRS.ADA.Assistants@aphis.usda.gov>
 Subject: FW: NEW FOIA: 2018-APHIS-00286

Hi Mildred,

Debra Donch said this one is for STAS because the records being requested are regarding a study that was done by WILDLIT which is under STAS, Beth's group.

Thanks!

From: Bundy, Mildred O - APHIS
 Sent: Monday, October 16, 2017 11:34 AM
 To: Fonville, Lisa F - APHIS <Lisa.F.Fonville@aphis.usda.gov>
 Cc: Bundy, Mildred O - APHIS <Mildred.Bundy@aphis.usda.gov>; Floyd, Rosalyn N - APHIS <Rosalyn.N.Floyd@aphis.usda.gov>
 Subject: NEW FOIA: 2018-APHIS-00286

Hey Lisa: This will go to Langston and Don Herriott

003072

All captured information has to been uploaded to the FOIA SharePoint site below:

<https://ems-team.usda.gov/sites/aphis-vs-oda/vsinfo/>

From: Frey, Rebecca K - APHIS
Sent: Wednesday, November 29, 2017 7:24 AM
To: Nol, Pauline - APHIS
Cc: McCollum, Matthew P - APHIS; Wehtje, Morgan E - APHIS
Subject: Re: bison

Not sure, but I think pretty good in this area which should get to the folks who like to call their congressman. That's the go to move for ranchers here.

Sent from my iPhone

On Nov 28, 2017, at 10:09 AM, Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov> wrote:
That was very well written.
How wide is the readership?

From: Frey, Rebecca K - APHIS
Sent: Tuesday, November 28, 2017 8:03 AM
To: McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>
Subject: Fwd: bison

Sent from my iPhone

Begin forwarded

Article from this week's Western Ag Reporter

From: Brent Thompson [mailto:(b) (6)@gmail.com]
Sent: Tuesday, November 28, 2017 7:42 AM
To: Thompson, Brent D - APHIS <Brent.D.Thompson@aphis.usda.gov>
Subject: bison

From: Rhyan, Jack C - APHIS
 Sent: Friday, December 15, 2017 4:31 PM
 To: Wehtje, Morgan E - APHIS; Robbe Austerman, Suelee - APHIS
 Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Held, Karl E - APHIS
 Subject: RE: Input for response letr

Y'all are the best!
 j

From: Wehtje, Morgan E - APHIS
 Sent: Friday, December 15, 2017 3:46 PM
 To: Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>
 Cc: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Held, Karl E - APHIS <Karl.E.Held@aphis.usda.gov>
 Subject: Re: Input for response letr

One other item is that the corwin springs gonacon study was approved under a Federal EA FONSI and is available on the USDAs web page under the Brucellosis section. Why would a federal agency willing advertise illegal activity.

Sent from my iPhone

On Dec 15, 2017, at 3:31 PM, Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov> wrote:
 Pauline, I agree with you.
 You all have been helpful. I have been so angry at this letter it has been difficult for me to put this stuff into words. We have to be polite and state our case clearly... that is not always easy for me. :(

From: Nol, Pauline - APHIS
 Sent: Friday, December 15, 2017 4:18 PM
 To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>; Held, Karl E - APHIS <Karl.E.Held@aphis.usda.gov>
 Subject: RE: Input for response letr

Therefore the statement implying that we "knowingly were in possession of a select agent" is completely false.
 This is also evidenced by our huge effort to acquire as much input as possible from all potentially involved agencies and institutions including the AgSAS regarding these studies.
 In my mind the fact that they put out the Position Statement in August of this year is frank admission to the lack of clarity in wording of the CFR.
https://www.selectagents.gov/policystatement_infectedanimals.html

From: Rhyan, Jack C - APHIS
 Sent: Friday, December 15, 2017 3:05 PM
 To: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>; Held, Karl E - APHIS <Karl.E.Held@aphis.usda.gov>
 Subject: RE: Input for response letr

Suelee,
 Let there be no mistake, as you know, our goal was to observe natural transmission such that we could prove out a model for testing vaccines using natural transmission instead of having to handle a select agent. We wanted to perfect a way to do vaccine studies in elk using naturally infected animals while being fully compliant with the regs as they existed and we understood them at the time. We found that natural transmission at our facility was very inefficient.
 Jack

From: Nol, Pauline - APHIS
 Sent: Friday, December 15, 2017 2:54 PM
 To: Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>;

Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; McCollum, Matthew P - APHIS
 <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS
 <Morgan.E.Wehtje@aphis.usda.gov>; Held, Karl E - APHIS
 <Karl.E.Held@aphis.usda.gov>
 Subject: RE: Input for response letr

I'm working on some more corrections.
 We had nonpregnant positive WY elk from 2016 co-mingled with seronegative elk at the time of Vicki's visit. None of the positive WY elk that came in 2017 were comingled with elk already on the property.
 All of the 2017 Wyoming elk regardless of Bruce status were housed together because we didn't know of their actual serostatus at the time of arrival.

From: Robbe Austerman, Suelee - APHIS
 Sent: Friday, December 15, 2017 2:49 PM
 To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS
 <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS
 <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS
 <Morgan.E.Wehtje@aphis.usda.gov>; Held, Karl E - APHIS
 <Karl.E.Held@aphis.usda.gov>
 Subject: RE: Input for response letr

Jack,
 The letter says something like they observed during the inspection that you all had elk mixed for the purposes to create transmission. That can't be correct. Did you have sero negative and sero positive animals together at the time of the visit?
 Thanks

From: Rhyan, Jack C - APHIS
 Sent: Friday, December 15, 2017 3:11 PM
 To: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS
 <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS
 <Morgan.E.Wehtje@aphis.usda.gov>; Held, Karl E - APHIS
 <Karl.E.Held@aphis.usda.gov>
 Cc: Robbe Austerman, Suelee - APHIS <Suelee.Robbe-Austerman@aphis.usda.gov>
 Subject: Input for response letr

Here are my thoughts. Please chime in, folks.
 Thanks,
 Jack

From: McCluskey, Brian J - APHIS
 Sent: Wednesday, January 24, 2018 12:07 PM
 To: Rhyan, Jack C - APHIS
 Cc: Nol, Pauline - APHIS
 Subject: RE: Responding to ITBC letter

Thanks Jack.

 Brian J. McCluskey, DVM, MS, PhD, Dip. ACVPM
 Associate Deputy Administrator
 Surveillance, Preparedness and Response Services
 USDA, APHIS, Veterinary Services
 970-494-7395

From: Rhyan, Jack C - APHIS
 Sent: Wednesday, January 24, 2018 12:06 PM
 To: McCluskey, Brian J - APHIS <brian.j.mccluskey@aphis.usda.gov>
 Cc: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
 Subject: FW: Responding to ITBC letter

Hey Brian,
 So as I recall we brought some of the research animals here in 2014 - 2016. They were our research animals (usually calves from the bison that were collected from the Park on a research collectors permit) from the GonaCon study that we wanted to monitor for a few years to answer questions about fertility in offspring of GonaCon treated animals. Additionally, many were used in further research on vaccination using DryDarts. We never brought animals captured in the trap here immediately for quarantine.
 Becky would probably have a better handle on which animals were sent and when.

Jack

From: Nol, Pauline - APHIS
 Sent: Wednesday, January 24, 2018 10:41 AM
 To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
 Subject: Fwd: Responding to ITBC letter

Jack, could you please assist?

Sent from my iPhone

Begin forwarded message:
 From: "McCluskey, Brian J - APHIS" <brian.j.mccluskey@aphis.usda.gov>
 Date: January 24, 2018 at 9:48:28 AM MST
 To: "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov>
 Subject: Responding to ITBC letter
 Hi Pauline,

I am assisting with a response to a letter from the ITBC regarding bison transfers. Hope you can answer a question for me. When were the Yellowstone bison moved from Corwin Springs to the foothills facility here in Fort Collins? The ITBC is unhappy that bison were moved to Colorado for quarantine and not to a tribal entity.

Thanks for your assistance.

Brian

 Brian J. McCluskey, DVM, MS, PhD, Dip. ACVPM
 Associate Deputy Administrator
 Surveillance, Preparedness and Response Services
 USDA, APHIS, Veterinary Services
 970-494-7395

From: Rhyan, Jack C - APHIS
Sent: Tuesday, November 19, 2013 11:38 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: sera

Our records show we received it in August. Matt is checking the freezer for it.
Thanks.
Jack

From: Nol, Pauline - APHIS
Sent: Monday, November 18, 2013 5:29 PM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS
Subject: RE: sera

I do not.

From: McCollum, Matthew P - APHIS
Sent: Monday, November 18, 2013 3:08 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: sera

Either of yous two know?
M

From: Frey, Rebecca K - APHIS
Sent: Monday, November 18, 2013 2:05 PM
To: McCollum, Matthew P - APHIS
Subject: sera

Hey, Did Ryan send you any of the serum from the GonaCon cows for gonacon titers this summer? If so, did you get the results?

Thanks
Killed a cow and 3 does so far.....got meat?

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: McCollum, Matthew P - APHIS
 Sent: Monday, November 27, 2017 1:26 PM
 To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; Wehtje, Morgan E - APHIS
 Subject: RE: USDA Cutbacks Include Decommissioning Brucellosis Studies

I'm expecting a call at any time from the administrator begging us to reconsider our decision to stop the research after we were directed to stop the research.

From: Nol, Pauline - APHIS
 Sent: Monday, November 27, 2017 10:23 AM
 To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>
 Subject: RE: USDA Cutbacks Include Decommissioning Brucellosis Studies

The last sentence should have added, "...the USDA Veterinary Services brucellosis research group that is now decommissioned."

From: Rhyan, Jack C - APHIS
 Sent: Monday, November 27, 2017 10:17 AM
 To: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>
 Subject: FW: USDA Cutbacks Include Decommissioning Brucellosis Studies

Word gets out.
 Jack

From: Bauer, Nathan - FSIS
 Sent: Monday, November 27, 2017 9:27 AM
 To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>
 Subject: USDA Cutbacks Include Decommissioning Brucellosis Studies

This is almost a week old but I just saw it today.
 Nate

USDA Cutbacks Include Decommissioning Brucellosis Studies
 By Traci Eatherton Tri-State Livestock News November 22, 2017

The U.S. Department of Agriculture's plans to decommission brucellosis field studies is in direct conflict of a recent study that concluded that brucellosis is spreading in wildlife, and more research is needed, not less, in both elk and bison.

The National Academy of Sciences (NAS) report, Revisiting Brucellosis in the Greater Yellowstone Area (GYA), published in May 2017, funded by APHIS (Animal & Plant Health Inspection Service) states, "top priority should be placed on research to better understand brucellosis disease ecology and epidemiology in elk and bison," and "the current spread of brucellosis will have serious future implications if it moves outside of the GYA."

The research is important, especially for producers in Montana, Idaho, and Wyoming. The National Assembly of State Animal Health Officials (NASAHO), has weighed in on the value of the research, calling USDA's plan deeply troubling.

Findings from prior research efforts have directly affected decisions relating to management of brucellosis, according to NASAHO president and veterinarian, Susan, Keller.

Studies on time management following land use in infected areas, remote vaccination of wildlife, and the ability bull bison to transmit brucellosis are all potentially on the chopping block.

Full text:
<https://www.tsln.com/news/usda-cutbacks-include-decommissioning-brucellosis-studies/>

Nathan E. Bauer, Jr., DVM, MS
 Veterinary Medical Officer, Science Staff, Office of Public Health Science , USDA, Food Safety and Inspection Service Co-Located with, USDA, ARS Food & Feed Safety Research Unit; 2881 F&B Road; College Station, TX 77845, Telephone Number: (979) 260-9409, Fax Number: (979) 260-9332; nathan.bauer@fsis.usda.gov

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: keith.roehr@state.co.us; [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: Re: 1-27 and CVI for bison from MT to CO
Date: Thursday, January 15, 2015 1:19:32 PM
Attachments: [ATT00001.txt](#)

Colorado import permit number attached.

Sent from my iPhone

> On Jan 15, 2015, at 11:17 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

>

> Apologies. They are now attached.

> [image.jpeg]

> Sent from my iPhone[image.jpeg]

>

> Begin forwarded message:

>

> From: <Pauline.Nol@aphis.usda.gov<mailto:Pauline.Nol@aphis.usda.gov>>

> Date: January 15, 2015 at 9:55:40 MST

> To: Keith Roehr <keith.roehr@state.co.us<mailto:keith.roehr@state.co.us>>

> Cc: Jack C - APHIS Rhyan <Jack.C.Rhyan@aphis.usda.gov<mailto:Jack.C.Rhyan@aphis.usda.gov>>, MATTHEW MCCOLLUM <Matt.McCollum@aphis.usda.gov<mailto:Matt.McCollum@aphis.usda.gov>>, Rebecca K - APHIS Frey <Rebecca.K.Frey@aphis.usda.gov<mailto:Rebecca.K.Frey@aphis.usda.gov>>, "Patrick R. - APHIS Clarke" <Patrick.R.Clarke@aphis.usda.gov<mailto:Patrick.R.Clarke@aphis.usda.gov>>

> Subject: 1-27 and CVI for bison from MT to CO

>

> Keith,
> Attached are the documents for the 10 bison we are bringing back to CO today. They are brucella -exposed but negative on serology. Please let me know if you cannot read them.

> Thanks!

> Pauline

>

> Sent from my iPhone

> <image.jpeg>

> <image.jpeg>

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: keith.roehr@state.co.us; [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: Re: 1-27 and CVI for bison from MT to CO
Date: Thursday, January 15, 2015 9:07:51 PM
Attachments: [ATT00001.txt](#)

Animals delivered. All got off the trailer fine.

Thanks much,
Matt

Sent from my iPhone

> On Jan 15, 2015, at 11:17 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

>

> Apologies. They are now attached.

> [image.jpeg]

> Sent from my iPhone[image.jpeg]

>

> Begin forwarded message:

>

> From: <Pauline.Nol@aphis.usda.gov<mailto:Pauline.Nol@aphis.usda.gov>>

> Date: January 15, 2015 at 9:55:40 MST

> To: Keith Roehr <keith.roehr@state.co.us<mailto:keith.roehr@state.co.us>>

> Cc: Jack C - APHIS Rhyan <Jack.C.Rhyan@aphis.usda.gov<mailto:Jack.C.Rhyan@aphis.usda.gov>>, MATTHEW MCCOLLUM <Matt.McCollum@aphis.usda.gov<mailto:Matt.McCollum@aphis.usda.gov>>, Rebecca K - APHIS Frey <Rebecca.K.Frey@aphis.usda.gov<mailto:Rebecca.K.Frey@aphis.usda.gov>>, "Patrick R. - APHIS Clarke" <Patrick.R.Clarke@aphis.usda.gov<mailto:Patrick.R.Clarke@aphis.usda.gov>>

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> Pauline

>

> Sent from my iPhone

> <image.jpeg>

> <image.jpeg>

PART 1 - TO ACCOMPANY SHIPMENT

Previous edition may be used.

VS FORM 1-2

[illegible]

Tel No 946-388-5162

Montana Department of Livestock
State Veterinarian
PO Box 202001, Helena, MT 59620-2001

MONTANA CERTIFICATE OF VETERINARY INSPECTION

81 - 454416

CONSIGNOR NAME AND ADDRESS
USDA, APHIS - GonaCon Study
Cotwin Springs, MT

CONSIGNEE NAME AND ADDRESS
USDA, APHIS Research Pens
4101 LaPorte Ave, Ft Collins, CO

PERMIT NO.
DATE ISSUED **15 Jan 15**
DATE RECD **15 Jan 15**
NO OF ANIMALS IN SHIPMENT **10**

PURPOSE OF MOVEMENT:
☐ BREEDING ☐ SLAUGHTER ☐ FEEDING ☒ RESEARCH
EXHIBITION, ETC.
SPECIES: ☐ CATTLE ☐ HORSES ☐ SHEEP ☐ SWINE ☐ POULTRY
☒ OTHER: **Bison**
ORIGIN OF SHIPMENT: **Park**
A) County: B) Market:

AREA OF ORIGIN STATUS:
☐ TB MODIFIED ACCREDIT
☐ TB FREE
☐ BRUCELLOSIS FREE
☐ PRV STAGES
☒ OTHER: **DSA**

CARRIER:
☒ TRUCK ☐ OTHER
NAME & ADDRESS: **USDA, APHIS**
4101 LaPorte Ave
Ft Collins, CO

VACCINATION OR TREATMENT FOR
(EXCEPT BRUCELLOSIS)
PRODUCT
DATE
RECORD NEGATIVE TEST RESULTS
LAB:

EAR TAG NO. TATTOO OR OTHER PERMANENT IDENTIFICATION	LINE NO.	REGISTRATION NAME AND NUMBER OR DESCRIPTION	VACCINATION TATTOO SYMBOL OR DATE	AGE	SEX	BREED	Disease Type of Test	Disease Type of Test
840-003-003-405-600	1	Burple tag Red 52	N/A	34	F	Bison		
607	2	Green 27		34	F			
608	3	Red 421		34	F			
609	4	Green 26		34	F			
610	5	Green 23		34	F			
840-003-003-341-951	6	LR03		4M	M			
956	7	4R07		4M	M			
957	8	4R16		4M	F			
958	9	4R21		4M	F			
952	10	4224		4M	F			
	11							
	12							
	13							
	14							
	15							
	16							

VETERINARY CERTIFICATION:
I certify as an Accredited Veterinarian that the above described animals have been inspected by me and that they are not showing signs of infectious, contagious, or communicable disease (except as noted). The vaccinations and results of tests are as indicated on the certificate. To the best of my knowledge the animals shown on this certificate meet State of Destination and Federal Interstate requirements. No warranty is made or implied.
Date: **15 Jan 15** Accredited Veterinarian Signature: **CLARKE**
Printed L: **(b) (6)**
Address: **(b) (6)**

OWNER/AGENT STATEMENT (where applicable)
The animals in this shipment are those certified to and listed on this certificate.
Signature: **(b) (6)**
Address: **(b) (6)**
Date: **15 Jan 15**

Form SV-7 (Rev. 8/99)

From: **Matthew McCollum**
To: **Matthew McCollum**
Cc: **Matthew McCollum**
Date: **1/15/2015**
Subject: **Montana Department of Livestock - State Veterinarian - PO Box 202001, Helena, MT 59620-2001**

From: [Rebecca K Frey](#)
To: [Jack C Rhyan](#); [Patrick R Clarke](#)
Cc: [Pauline Nol](#); [Matt McCollum](#)
Subject: Re: ACUC Proposal - signed
Date: Monday, May 16, 2011 4:42:00 PM

Yes, let's get to committee right away.
Becky Frey

From: Jack C Rhyan
Sent: 05/16/2011 06:08 PM GMT
To: Patrick Clarke
Cc: Pauline Nol; Rebecca Frey; Matt McCollum
Subject: ACUC Proposal - signed

Ryan,

Attached is the signed copy of the proposal for the ACUC. I made three changes. I said we would sample the bison 3 times a year instead of 2. I figured we would use the old scheme we used on the pathogenesis and epi study in the park, Feb, at calving, and in the fall. Since the study has developed from a low maintenance study to a more science-based study, I thought it worth the extra effort. Also, Lowell and I talked about the injection and we agreed 3000 microliters in 3 mls vaccine delivered in split injections on either side of the neck. The GonaCon is produced at 1000 ul per ml so it would be good to use the standard concentration, and I worried about that much adjuvant at one site causing marked injection site reactions. Also Lowell is doing a cattle study in Australia with the same protocol.

And finally, I talked with Freeda Isaac and she said now the way they are interpreting the select agent rules, we will be able to sample culture-positive animals repeatedly. Whew!

If any of you want to change it, please let me know. I can make changes and resign and send it. Otherwise, Ryan, I think it is ready for the Committee's scrutiny.

Jack

From: [Nol, Pauline \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#)
Subject: RE: ACUC
Date: Monday, May 23, 2011 4:22:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011final5.23.11.docx](#)

From: Rhyan, Jack C (APHIS)
Sent: Monday, May 23, 2011 4:08 PM
To: Nol, Pauline (APHIS)
Subject: ACUC

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <p>_____</p> <p style="text-align: left;">Permit(s) description Number Date</p>

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: _____Live animal work_____*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator

Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle

(Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute.

Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain

negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
 Xylazine- 0.07 mg/kg, IM dart

 Carfentanil-0.005-0.01 mg/kg, IM dart
 Xylazine- 0.07 mg/kg, IM dart

 Butorphenol- 0.03-0.06 mg/kg, IM dart
 Medetomidine- 0.01-0.02 mg/kg
 Azaperone- 0.02 mg/kg

Reversal for narcotics:

 Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
 Tolazoline-300 mg as needed IM

Reversal for BAM:

 Atipamezole 0.0375-0.03 mg/kg IM
 Naltrexone 0.05-0.125mg/kg IM
 Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyon, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

SIGNATURE PAGE

Study Director _____ Date_____

Concur

IACUC Chair _____ Date_____

From: [Rhyan, Jack C \(APHIS\)](#)
To: [Stephens, Stephanie H \(APHIS\)](#); [Eisemann, John D \(APHIS\)](#)
Cc: [Nol, Pauline \(APHIS\)](#)
Subject: RE: amendment document for the IACUC
Date: Monday, July 11, 2011 1:31:04 PM

Stephanie,

That sounds great. John said you and he would also send a letter to EPA asking them about the necessity of an EUP and getting some commitment on paper as to whether or not we need one. Good strategy.

Thanks much for all your work on this.

Jack

From: Stephens, Stephanie H (APHIS)
Sent: Monday, July 11, 2011 1:27 PM
To: Rhyan, Jack C (APHIS); Eisemann, John D (APHIS)
Subject: RE: amendment document for the IACUC

Jack-

Excellent, thank you. Assuming this is the final protocol, we are getting going on writing the EA right away.

As soon as we have our internal EA team meeting (scheduled for Wednesday of this week), I'll call you to discuss the schedule/deadlines for the EA so you're aware of the final plan.

Thanks,

Stephanie

From: Rhyan, Jack C (APHIS)
Sent: Friday, July 08, 2011 1:35 PM
To: Eisemann, John D (APHIS); Stephens, Stephanie H (APHIS)
Subject: FW: amendment document for the IACUC

John and Stephanie,

Here are the amendments including the 3rd objective about efficacy. I also attached the previous protocol.

Jack

From: Nol, Pauline (APHIS)
Sent: Friday, July 01, 2011 2:47 PM
To: Rhyan, Jack C (APHIS)
Subject: amendment document for the IACUC



This will be attached to the original document after approval.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Thompson, Brent D - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: an enigma, wrapped in a puzzle
Date: Tuesday, October 14, 2014 2:13:54 PM

So let's try to organize this call. I good any time this week except for Friday after ~11 am. What works for everyone else?

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Monday, October 06, 2014 3:59 PM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Cc: Thompson, Brent D - APHIS
Subject: RE: an enigma, wrapped in a puzzle

"Reproduction is a powerful force!" – Keith Aune, circa 2001.

Looking forward to the call.

Jack

From: Frey, Rebecca K - APHIS
Sent: Monday, October 06, 2014 12:16 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Cc: Thompson, Brent D - APHIS
Subject: an enigma, wrapped in a puzzle

Soooooooo, it would seem that bison bulls are even a larger pain in the rump than originally thought! On the night of September 23rd, the leftover bulls, got in with the leftover cows(which will be the 2nd control pen after we get a few more infected cows). We got them sorted out the evening of the 24th.....but since there were 5 bulls with about 12 cows, it could be one or more that got bred. Brent says 35 days would be ideal to ultrasound and lutalyse pregnant cows. How do you feel about the consequences to the "controls" if they have been bred, and if we lutalyse them, or would you prefer to lutalyse all of them? I think it would be very good to try and determine which ones/how many were bred either way. Do we remove anybody that got pregnant no matter what? Lots of questions..... Please think on this and we will try to have a call next week after Clarke is back from vacation.

35 days puts us at October 29th....for which I have annual leave....I am back on the 30th? We can get Steve to bring the ultrasound up on his next trip from CO.

Thanks,
Becky

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Thompson, Brent D - APHIS](#)
Subject: RE: an enigma, wrapped in a puzzle
Date: Tuesday, October 14, 2014 3:17:56 PM

Heard from everyone.

Let's make it 2 pm tomorrow, Wednesday the 15th

I'll initiate the call.

Call in #: (b) (6)

Code: (b) (6)

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Tuesday, October 14, 2014 2:54 PM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Thompson, Brent D - APHIS
Subject: RE: an enigma, wrapped in a puzzle

Wednesday afternoon is good. Say 2pm?

From: Nol, Pauline - APHIS
Sent: Tuesday, October 14, 2014 2:37 PM
To: McCollum, Matthew P - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Thompson, Brent D - APHIS
Subject: RE: an enigma, wrapped in a puzzle

I'm only around Wednesday afternoon. Thursday and Friday not good but I'm sure you can fill me in.

From: McCollum, Matthew P - APHIS
Sent: Tuesday, October 14, 2014 2:30 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Thompson, Brent D - APHIS; Nol, Pauline - APHIS
Subject: RE: an enigma, wrapped in a puzzle

Thursday any time looks best for me. Also Wednesday afternoon.

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, October 14, 2014 2:14 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Thompson, Brent D - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

Subject: RE: an enigma, wrapped in a puzzle

So let's try to organize this call. I good any time this week except for Friday after ~11 am. What works for everyone else?

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Monday, October 06, 2014 3:59 PM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Cc: Thompson, Brent D - APHIS
Subject: RE: an enigma, wrapped in a puzzle

"Reproduction is a powerful force!" – Keith Aune, circa 2001.
Looking forward to the call.
Jack

From: Frey, Rebecca K - APHIS
Sent: Monday, October 06, 2014 12:16 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Cc: Thompson, Brent D - APHIS
Subject: an enigma, wrapped in a puzzle

Sooooooooo, it would seem that bison bulls are even a larger pain in the rump than originally thought! On the night of September 23rd, the leftover bulls, got in with the leftover cows(which will be the 2nd control pen after we get a few more infected cows). We got them sorted out the evening of the 24th.....but since there were 5 bulls with about 12 cows, it could be one or more that got bred. Brent says 35 days would be ideal to ultrasound and lutalyse pregnant cows. How do you feel about the consequences to the "controls" if they have been bred, and if we lutalyse them, or would you prefer to lutalyse all of them? I think it would be very good to try and determine which ones/how many were bred either way. Do we remove anybody that got pregnant no matter what? Lots of questions..... Please think on this and we will try to have a call next week after Clarke is back from vacation.

35 days puts us at October 29th....for which I have annual leave....I am back on the 30th? We can get Steve to bring the ultrasound up on his next trip from CO.

Thanks,
Becky

Rebecca Frey
Wildlife Disease Specialist

USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: Another long term study question.....
Date: Friday, February 06, 2015 1:18:35 PM

I'd say keep it in your stash but let's delete it. No results are better than bad results!

j

From: Frey, Rebecca K - APHIS
Sent: Friday, February 06, 2015 12:05 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: Another long term study question.....

Sorry, I have just been working with the database a lot lately.....anyway, we started the GC project using Rivanol as one of the regular tests, however, the reagents have been off..(a known lab issue)... and we have been getting some bizarre results from Rivanol....almost everybody has rivanol titer at +50 or more. Soooooo, we have quit....at labs discretion....using Rivanol. They and we felt it was not telling us anything anyway. I have no idea if they will resolve the rivanol issue before we finish this study. Presumably they will, but who knows. That being said, what do we plan to do with the Rivanol test results, and if we don't have a complete set of tests over the years as with FP and CF and others....do we want to keep that data at hand or ignore it? I plan to "hide" that column for now....but I may delete in future.....we still have all of the paper results filed away in my most secret GC stash. ☺

Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Laura B Greiner](#)
To: [Pauline Nol](#)
Subject: Re: another question
Date: Monday, March 07, 2011 12:47:00 PM
Attachments: [QA-1523 Gionfriddo Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk at RMNP.pdf](#)
[SOP Index - 9 November 2010.doc](#)

Pauline,

The elk protocol is attached as a pdf. I've also attached the current list of SOPs. I can send you the files for any that look relevant. Let me know if you have any other questions.

Laura

(See attached file: QA-1523 Gionfriddo Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk at RMNP.pdf)(See attached file: SOP Index - 9 November 2010.doc)

☐ Pauline Nol---03/07/2011 12:30:58 PM---Hi Laura and Cathy, So I'm writing up this protocol to do GonaCon work in bison up in Montana. Well I guess I have to bug you

**Pauline
Nol/CO/APHIS/USDA**

ToCatherine M Bens/CO/APHIS/USDA@USDA,
Laura B Greiner/CO/APHIS/USDA@USDA

cc

03/07/2011 12:30 PM

Subjectanother question

Hi Laura and Cathy,

So I'm writing up this protocol to do GonaCon work in bison up in Montana. Well I guess I have to bug you again since I have to do an SOP search and have no idea which SOP's would be related to the study. I'm sure this was covered in your training (that I missed!) so I apologize for the repetition. And once again, I guess it would help me to be able to get on the intranet!!

Also, How do I get my hands on the protocol on Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado? I believe that was a GLP study as well and I think my protocol will have a lot of similarities to that one.

Thanks for your help!!

Pauline

**National Wildlife Research Center
Wildlife Services
Animal and Plant Health Inspection Service
United States Department of Agriculture**

Study Protocol

1. Title: Field Study of GonaCon™ Immunocontraceptive Vaccine for Use in the Contraception of Elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado.

2. Study Director: James P. Gionfriddo

3. Sponsor:

Name: United States Department of Interior
National Park Service
Biological Resource Management Division
Margaret A. Wild
1201 Oak Ridge Drive, Suite 200
Fort Collins, Colorado 80525
Telephone: (970) 225-3593

Name: United States Department of Agriculture
Animal and Plant Health Inspection Service
National Wildlife Research Center
Kathleen A. Fagerstone
Research Program Manager, ISTRD Program
4101 LaPorte Avenue
Fort Collins, Colorado 80521-2154
Telephone: (970) 266-6161

4. Test Sites and Testing Facility:

Field Test Site:

Name: Rocky Mountain National Park (Dr. Margaret Wild)
Address: 1201 Oak Ridge Drive, Suite 200
Fort Collins, Colorado 80525

Test Site, Johne's Disease Analysis:

Name: Colorado Department of Agriculture's Rocky Mountain Regional Animal Health Laboratory
Address: 2331 West 31st Avenue
Denver, Colorado 80211

Test Site, Blood Serum Pregnancy (PSPB) Analysis:

Name: Bio-Tracking
Address: 1150 Alturas Drive, Suite 105
Moscow, Idaho 83843

Test Site, Necropsies and Histopathological Analysis:

Name: Colorado State University Veterinary Diagnostic Laboratory (Dr. Terry Spraker)
Address: 300 West Drake Road
Fort Collins, Colorado 80523

Test Facility (source of test material [GonaCon[™] vaccine], GLP [Good Laboratory Practices] compliance, and preparation of final report on GonaCon[™] for submission to the U.S. EPA):

Name: National Wildlife Research Center
Address: 4101 LaPorte Avenue
Fort Collins, Colorado 80521

Note: Three of the above test sites (the Colorado Department of Agriculture's Rocky Mountain Regional Animal Health Laboratory, Bio-Tracking, and the Colorado State University Veterinary Diagnostic Laboratory) are not GLP (Good Laboratory Practices) -compliant laboratories. Assays at these laboratories will not be conducted under GLP standards. Data to be analyzed at the Colorado Department of Agriculture's Rocky Mountain Regional Animal Health Laboratory and at Bio-Tracking will be supplemental data.

5. Background and Justification:

National Park Service (NPS) natural resource managers at Rocky Mountain National Park (RMNP) in Colorado have offered scientists at the National Wildlife Research Center (NWRC) an opportunity to evaluate the efficacy of GonaCon™ Immunocontraceptive Vaccine as a contraceptive agent in overabundant elk at RMNP. NWRC will provide the vaccine that NPS and CSU (Colorado State University) scientists will test in the field. NPS/CSU scientists will evaluate GonaCon™ as a temporary infertility agent in free-ranging adult female elk that are targeted for subsequent lethal removal under a separate NPS population reduction program.

This NWRC QA (quality assurance) study is part of the attached NPS study plan entitled "Evaluation of Methods for Managing Elk Population Health and Abundance in Rocky Mountain National Park, Colorado" (Appendix 29.1). In this subpart of the NPS study plan, a 3-year field study of contraceptive efficacy will be conducted at RMNP. About 60 elk cows will be captured, fitted with radio telemetry transmitters, vaccinated with GonaCon™, and then released. An additional 60 cows will be captured, radio-collared, given a "sham" injection (consisting of sterile saline solution), and then released as control animals. NPS/CSU will provide a field principal investigator, field personnel, delivery of the test material (vaccine) to the elk, all animal handling and monitoring, and data collection. All field research activities will be conducted by NPS/CSU; NWRC will not participate in field activities. NWRC will provide the immunocontraceptive vaccine, give limited technical assistance, and provide GLP oversight for the study.

6. Objective/Hypotheses:

NWRC's QA study is to be conducted in support of the NPS/CSU study plan (Appendix 29.1). These data will be used in support of registration should Wildlife Services decide to proceed with registration of GonaCon™ Immunocontraceptive Vaccine with the EPA. The formulation used in this study will be the formulation for any possible future registration (see Appendix 29.3, "Test, Control and Reference Material/Devices Formulation and Use Appendix"). The objective of the research under this protocol is to confirm the effectiveness of GonaCon™ in reducing reproduction by female elk under field-use conditions. The efficacy of a single injection of GonaCon™ will be determined by monitoring the reproductive success of 60 treated females over part or all of a three-year period.

7. NWRC Approved Project Title: Development of Reproductive Control Methods for Overabundant Birds and Mammals

8. Regulatory Compliance/Guidelines:

Indicate the regulatory standard or test guidelines under which this study will be conducted.

Regulatory Standard: Check as appropriate.

<input type="checkbox"/>	None, non-regulated study
<input checked="" type="checkbox"/>	CFR Title 40, Part 160: Good Laboratory Practice Standards (FIFRA);
<input type="checkbox"/>	CFR Title 21, Part 58: GLP Standards for Nonclinical laboratory Studies, (FFDCA)
<input type="checkbox"/>	Other:

9. Study Classification Information

Does this study include any or all of the following? Check as appropriate.

<input checked="" type="checkbox"/>	Animals -- please complete and attach Animal Use Appendix
<input type="checkbox"/>	Plants -- no additional appendix required
<input type="checkbox"/>	Microbiological/Biohazardous Materials -- please complete and attach Microbiological/Biohazardous Materials Use Appendix
<input type="checkbox"/>	Chemical Analysis -- please complete and attach Analytical Chemistry Appendix
<input type="checkbox"/>	Literature review only -- no additional appendix required
<input type="checkbox"/>	Statistical or economic analysis only -- no additional appendix required
<input checked="" type="checkbox"/>	Use of a test, control, references substance, bait or device -- complete and attach Test, Control and Reference Materials / Device Formulation and Use Appendix

10. Methods/Procedures:**10.1 General**

Located in north-central Colorado, about 60 miles to the northwest of Denver, the study area encompasses the elk winter range within and near the eastern portion of Rocky Mountain National Park (RMNP) (Figure 1). All elk to be included in this contraception study will be captured within the national park.

Federal legislation and policy require the NPS to manage its lands and waters so as to preserve natural and other resources in an unimpaired condition for the benefit of current and future generations of U.S. citizens (USDI NPS 2001). The NPS is specifically mandated to maintain and restore, to the extent possible, the natural conditions and processes in park units such as RMNP (USDI NPS 2006). To assist the NPS in the management of natural resources at RMNP so as to achieve these objectives, an elk and vegetation management plan was developed. In April 2006, the NPS completed a draft environmental impact statement (EIS) and elk and vegetation management plan for Rocky Mountain National Park (USDI NPS 2006). The document evaluated 5 "action alternatives" or strategies for managing resident populations of elk, aspen, willow, and other species of vegetation. It also evaluated the anticipated impacts of implementing each of the proposed management strategies. Of the 5 management alternatives identified and described in that plan,

alternative 3, which involves the gradual lethal removal of elk, was selected as the preferred alternative. Under this management alternative, the RMNP elk population will be reduced over a 20-year period via culling of animals by NPS staff or contractors.

The lethal removal of elk via sharpshooting, which will be initiated during 2008, is beyond the scope of this NWRC research protocol. The use of lethal control to reduce elk densities at RMNP, however, provides an opportunity to test GonaCon[™] Immunocontraceptive Vaccine in elk under field conditions. This protocol describes the NWRC oversight of the contraceptive field research activities that NPS/CSU will conduct at RMNP.

During early 2008 (approximately January 3 through March 15), NPS/CSU researchers will drive along park roads in search of groups of elk. When elk are located, observations of individual animals will be used to evaluate the elk for visual clinical signs of chronic wasting disease (CWD). Selected elk that are determined to be free of visual clinical signs of CWD will be captured for inclusion in the contraception study. Clinical signs of CWD include poor body condition, lowered head/ears, salivation, abnormal behavior, head tremors, and remaining near water sources for extended periods. About 120 adult (≥ 2 years old) female elk will be captured, marked, and fitted with radio telemetry collars. A tranquilizer dart containing a mixture of 2.7 mg of carfentanil (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and 10 mg of xylazine (Tranquived, Vedco, St. Joseph, Missouri, USA) will be used to capture and immobilize each elk. A CO₂ rifle (Dan-Inject[™], Dan-Inject of North America, Fort Collins, Colorado, USA) will be used to deliver the barbed, 1- or 2-cc self-injecting tranquilizer darts. Tranquilizer darts will be fired from vehicles or from the ground, and generally will be fired from distances of 35 meters or less. Captured elk will be randomly assigned to treatment and control groups. Half (about 60) of the captured elk will receive a single intramuscular injection of GonaCon[™] Immunocontraceptive Vaccine. Each vaccine dose will contain 2,000 μ g of GnRH-blue protein conjugate emulsified in AdjuVac[™] adjuvant. The remaining (about 60) captured female elk will serve as control animals; each will receive a 2-cc injection of sterile saline solution. All (vaccine and saline solution) injections will be administered to the hip by hand-held syringe. When elk are eventually collected and necropsied, the injection sites must be identified accurately so that tissue samples may be taken for gross examination and histopathological analysis to determine the presence and nature of localized tissue reactions to vaccination. To facilitate the subsequent relocation of injection sites (at necropsy), the capture team will document, at the time of initial elk capture in 2008, the specific locations of injection sites through measurements and reference to anatomical features.

If captured female elk are determined to be malnourished or obviously injured, they will not be considered for assignment to either (treatment or control) group, and such animals will be released or they will be euthanized by a gunshot to the head or by the injection of an overdose of barbiturates. Captured elk that are obviously injured or that possess visible injuries or deformities which, in the experience of the veterinarian or trained examiner, require confinement and/or additional treatment; will be cared for before being released or they will be euthanized by a gunshot to the head or by the injection of an overdose of barbiturates. Any elk that tests positive for CWD on either rectal biopsy (as part of a related study that is not covered by this NWRC protocol) or blood assays will be subsequently removed from the population by lethal means and then submitted for necropsy at the Colorado State University Veterinary Diagnostic Laboratory. Any elk that are removed from



the study before March 15, 2008 will be replaced via the capture and processing of additional animals.

All capture and immobilization operations during the proposed field study will be supervised by Margaret A. Wild, a wildlife veterinarian with the NPS who has had extensive experience in the capture and immobilization of ungulates at RMNP. Vital signs will be monitored throughout the animal handling phase of the capture-related field activities to ensure animal safety and welfare. Blindfolds will be used to calm all captured elk. If elk experience wounds at the sites of tranquilizer dart entry, such wounds will be treated topically with antibiotic ointment. Animal age will be estimated via observation of tooth replacement and wear patterns (Quimby and Gaab 1957). A blood sample (200 ml) will be collected from the jugular vein of each elk, and an assessment of general body condition (Riney 1960) will be made. A portion of each blood sample will be submitted to the Colorado Department of Agriculture's Rocky Mountain Regional Animal Health Laboratory for analysis for the presence of antibodies to John's disease. A second portion of each blood sample will be submitted to BioTracking (Moscow, Idaho, USA) for pregnancy analysis via the evaluation of pregnancy-specific protein B (PSPB) in serum (Noyes et al. 1997). Pregnancy status of experimental elk also will be determined in the field via rectal palpation of the fetus, fetal membranes, or uterine cotyledons (Greer and Hawkins 1967). Additional portions of the original 200-ml blood sample may be analyzed by other wildlife researchers as they conduct research unrelated to this contraception study.

Each experimental elk will be fitted with a VHF radio telemetry collar (Advanced Telemetry Systems, Isanti, Minnesota, USA) and injected with GonaCon™ vaccine or sterile saline solution before being released at the capture site. Each radio telemetry collar will be sheathed by a plastic identification sleeve marked with a unique alpha-numeric code in 76-mm-tall black letters on a colored background (yellow for GonaCon™-treated elk and blue for control elk) (Freddy 1993). Each radio telemetry collar affixed to a GonaCon™-treated elk will bear the message "DO NOT CONSUME" and NPS contact information. Each radio telemetry collar affixed to a control elk will bear the message "DO NOT CONSUME IF KILLED BEFORE [a date at least 45 days after the capture date will be inserted here]" and NPS contact information. (The 45-day delay between the date of elk capture and the earliest date of consumption of the elk by humans will permit the clearance of carfentanil from the elk's body.) If an elk is captured but is not included in the contraceptive study (e.g., due to minor injuries or yearling age status), she will be euthanized with a gunshot to the head at close range or with an injection of an overdose of barbiturates, or she will be individually marked with a colored, numbered ear tag and released. In such cases, which are expected to be very rare, the ear tag will also bear the message "DO NOT CONSUME IF KILLED BEFORE [a date at least 45 days after the capture date will be inserted here]" and NPS contact information. Before being released, every captured elk will be given a subcutaneous dose (6,000,000 IU) of long-acting penicillin (Durapen, Vedco, St. Joseph, Missouri, USA) to reduce the possibility of capture-related infection.

Experimental elk will not be monitored via radio telemetry as part of this study. Each radio transmitter, however, will have a mortality switch that will be activated if the elk does not move for more than 4 hours, or if the transmitter falls off and is motionless for more than 4 hours. If NPS/CSU technicians or volunteers are available to monitor the survival of experimental elk via radio telemetry, then NWRC will be notified if mortality is discovered.

The reproductive status of a subset (sample) of the surviving vaccinated and control elk will be determined by NPS/CSU between January 1 and February 15 each year during the period 2009-2011. Each year, approximately 40 experimental elk (20 GonaCon™-treated animals and 20 control animals) will be lethally shot by certified sharpshooters as part of the NPS lethal elk removal operations. Postmortem physical examinations of the carcasses of these 40 elk will be conducted, and the pregnancy status of each animal will be determined via inspection of the reproductive tract for the presence of a fetus. In addition, the ovaries, uterus, anterior pituitary gland, hypothalamus, and tissue from the injection site (and its associated intramuscular vaccine depot site) will be collected and preserved for subsequent histopathological analysis. Necropsies will be conducted at the Colorado State University Veterinary Diagnostic Laboratory by Dr. Terry Spraker, a veterinary diagnostic pathologist. Dr. Spraker also will be responsible for oversight and reporting of results of the histopathological analyses.

To evaluate the effects of GonaCon™ Immunocontraceptive Vaccine on elk body condition, a body condition scoring system developed by Kistner (1980) will be used. This system estimates (1) fat in selected indicator sites (cardiac, omental, perirenal, and subcutaneous areas), and (2) the condition of skeletal muscle mass. These fat and condition indices provide a practical field technique for estimating body composition in elk carcasses (Cook et al. 2001a, b). Kistner's (1980) system will be used to evaluate body condition of culled and other experimental elk at necropsy.

During the course of this study, minor modifications may be made to the data forms to increase clarity and to facilitate the recording and interpretation of data. Any such changes to data forms must be approved by the study director.

10.2 Tissue Collection

Selected tissue samples are to be collected from elk carcasses during January and February of each year (2009-2011), when 40 experimental elk are lethally shot and collected by NPS/CSU. Tissues to be collected from each carcass for this study include the ovaries, uterus, anterior pituitary gland, hypothalamus, and injection site area. Tissue samples will be preserved in 10% buffered formalin or a similar preservative and analyzed for histopathology. Necropsy and histopathology reports will be completed and kept on file. Additional tissue samples will be collected from experimental elk for use in other research studies that, like this contraception study, will take advantage of the NPS's elk reduction operation. All of these research studies will "piggyback" on the NPS's elk management program by collecting data from animals that will be removed from the population under the park's elk management plan. The collection and analysis of elk blood and tissue samples for use in other research studies is not expected to have any impact on this contraception study.

10.3 Physical Description and Examination

Physical description and examination of study elk will include overall body condition, sex, estimates of body weight and age class, pregnancy status, obvious injuries and scars, and visible external parasites. Assessment of overall body condition of live elk at capture will follow the classification scheme described by Riney (1960). Assessment of overall body condition of culled and other elk at necropsy will follow the classification scheme described by Kistner (1980).

11. Experimental Design and Statistical Analyses:

11.1 General

Adult elk cows captured at RMNP during January-March 2008 will be randomly assigned to treatment groups (GonaCon™-treated and control groups). Control elk will be injected only with a sham material (sterile saline solution), and they will be used to establish a baseline normal level of reproduction for comparison with the GonaCon™-treated elk.

Each year (2009-2011) during the period January 1 through February 15, about 40 experimental elk (20 GonaCon™-treated animals and 20 control animals) will be collected by NPS/CSU via lethal gunshot with a high-powered rifle. The reproductive (pregnancy) status of each of these 40 elk will be determined via postmortem physical examination of the reproductive tract. The use of replicated vaccinated and control animals on the same study site will provide a direct evaluation of vaccine treatment efficacy.

A comparison of the proportion of vaccinated elk that become pregnant versus the proportion of control (unvaccinated) elk that become pregnant each year will provide the primary measure of vaccine efficacy for each year. The experimental units will be the individual elk in the treated (n ~ 60) and control (n ~ 60) groups. Reproductive data will be analyzed separately for each year to determine changes in vaccine efficacy over time. If the proportion of vaccinated elk that become pregnant is 75% lower than the proportion of control animals that do so, then the vaccine treatment will be considered efficacious, based on past performance of the contraceptive vaccine.

11.2 Variables Measured for Label Claim:

The following variables will be measured to evaluate the extent of contraception of vaccinated elk.

11.2.1 When and How Variables will be Determined to Support Label Claims and Equipment Used:

Variable	How Assessed	When Assessed	Equipment Used
proportion of treated elk that become pregnant	postmortem physical exam	January-February 2009-2011	observation

11.2.2 Calculations Using Data:

Objective measurements of the numbers of elk that become pregnant will be the only measurements used directly to calculate averages and standard deviations for vaccinated and control groups. Comparisons of these measurements will be used to determine the contraceptive effects of GonaCon™.

12. Description of Environmental Conditions and Monitoring Requirements:

This study will be conducted by CSU and NPS in the field under ambient conditions. General weather conditions and approximate air temperatures will be recorded in the field during field activities.

13. List number and title of Standard Operating Procedures (SOPs):

The following NWRC SOPs will be followed relative to NWRC's GLP oversight role in the study:

- AD 004.01 Archiving studies
- AD 007.01 Final reports
- AD 011.02 Data recording and error correction
- AD 012.02 Test, control, and reference substance chain of custody
- BT 016.02 Manufacture of GonaCon™ Immunocontraceptive Vaccine
- BT 017.00 (Draft) Enzyme-Linked Immunosorbant Assay (ELISA) method for assessing immune response to GnRH vaccine (deer)
- HS 004.00 Personal protective equipment

This protocol document contains sufficiently detailed descriptions of the procedures involved in capturing, marking, and injecting elk (with vaccine or saline solution) that an SOP describing these activities is deemed unnecessary, and therefore such an SOP is not included for this field study.

No SOPs are available for the necropsy procedures or for the histopathological analyses. Necropsies and histopathological evaluations are to be conducted under the supervision of Dr. Terry Spraker, a veterinary diagnostic pathologist at the Colorado State University Veterinary Diagnostic Laboratory (a non-GLP-compliant facility) in Fort Collins, Colorado. Several steps will be taken to mitigate the lack of SOPs for these procedures. Laboratory procedures will be inspected by the NWRC quality assurance officer. Necropsy and histopathology forms will be completed as the respective laboratory procedures are conducted. After they have completed several necropsies, the necropsy team will write a description of the procedure they have developed and used. This written description of the necropsy procedure will be kept on file and archived with the other records from this study. CVs will be kept on file at NWRC for Dr. Spraker and other involved laboratory staff, to document their expertise in the relevant scientific procedures and disciplines.

14. List of Records to be Maintained:

The protocol, all data, and all reports related to the contraception subpart of the study plan (Appendix 29.1) will be archived, retained, and maintained in the archives of NWRC under Study No. QA-1523. Data will include (1) records of animal capture, blood sampling, injection, and marking, (2) John's analysis records, (3) pregnancy-specific protein B analysis records, (4) records of results of rectal palpation to determine pregnancy status, (5)

necropsy records, (6) tissue sample collection records, (7) histopathology records, and (8) records of statistical analyses. The NWRC Archive is operated under EPA/FDA Good Laboratory Practices (GLPs). Additional records to be archived, retained, and maintained for this study include raw data sheets, protocol deviations and amendments, relevant correspondence, training records and CVs of field personnel, and chain-of-custody documents.

15. Permits/Certifications:

No collection permits will be needed, as all elk are to be captured within the national park by NPS/CSU personnel.

16. Endangered Species Act Compliance:

Is there a possibility that the study, as proposed, will or may affect threatened or endangered (T&E) species?

Yes: _____ No: X , this study will have no effect on any T&E species.

17. Historical Resources:

Does the study involve any major ground disturbance or otherwise have the potential to adversely affect historic resources?

Yes: _____ No: X

18. National Environmental Policy Act Compliance:

Does this study qualify for categorical exclusion¹ from further NEPA analysis?

Yes: X No: Unsure:

19. Employee and Public Safety:

The only portions of the contraceptive field study that will involve the potential for risk to the safety of employees or the public are the elk capture phase, when tranquilizer darts will be fired at selected elk, and the annual (January-February, 2009-2011) elk collection phase, when elk will be lethally shot with high-powered rifles. Risk will be greatly reduced via (1) carefully controlled access to the RMNP property, (2) warnings and other announcements to park employees and visitors that elk capture or collection is occurring and that capture or collection areas should be avoided, (3) the relative isolation of sites selected for elk capture and collection at RMNP, (4) the exercise of extreme caution by the wildlife professionals as they conduct capture and collection activities, and (5) the extensive training and experience that the capture and collection teams have accumulated.

20. Schedule:

Proposed Experiment Start Date: January 2008

(The Experiment Start Date is the date the test substance is first applied to the animals or test system or data are collected directly from the study)

¹ Categorical exclusion is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i) which categorically exclude:

"Research and development activities . . . that are carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal.

or at 7 CFR Part 372.5(c)(1)(i) which categorically exclude:

Routine measures, such as . . . surveys, sampling that does not cause physical alteration of the environment, testing . . . removals . . . (This) may include the (lawful) use . . . of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, provided that such use . . . : (A) . . . is localized or contained in areas where humans are not likely to be exposed, and is limited in terms of quantity . . . B) . . . will not cause contaminants to enter water bodies . . . (C) . . . does not adversely affect any federally protected species or critical habitat; and (D) . . . does not cause bioaccumulation.

Proposed Experiment Termination Date: April 2011
(The Experiment Termination Date is the last date on which data are collected directly from the study)

Proposed Study Completion/Archive Date: December 2012
(The study completion date is the date the final report is signed by the Study Director. Note that the study should be archived on this day)

21. Staffing:

NPS/CSU will provide a field principal investigator, field personnel, delivery of the test material (vaccine) to the elk, all animal handling and monitoring, and data collection. All field research activities will be conducted by NPS/CSU and will be coordinated by the principal investigator, Dan L. Baker. The NWRC will provide the GonaConTM Immunocontraceptive Vaccine, give limited technical assistance, and provide GLP oversight for the study. The NWRC will not participate in field activities.

USDA/NWRC:

Study Director (Wildlife Biologist)	0.12 FTE x 4 yrs = 0.48 FTE
Study Monitor (Quality Assurance)	0.04 FTE x 4 yrs = 0.16 FTE
Biological Technician (Laboratory)	0.04 FTE x 4 yrs = 0.16 FTE

As study director, James P. Gionfriddo will ensure that the NWRC protocol and any amendments are approved by the sponsors and made available to study personnel. He will also ensure that the procedures specified in the NWRC protocol and any amendments are followed, and that all raw data are fully documented and recorded.

22. Principal Investigators, Cooperators and Consultants:

Name: Colorado State University, Dept. of Biomedical Sciences (Dan L. Baker)
Address: Fort Collins, Colorado 80523

As principal investigator, Dan L. Baker will coordinate and oversee all field research activities. He will be responsible for the capture, handling, and experimental treatment of elk, as well as the final collection and disposition of experimental animals and the collection, preservation, and submission of biological (blood and tissue) samples for laboratory analysis. He will keep the study director informed of the progress of field activities, and of the disposition (e.g., mortality, disappearance, final collection) of experimental elk throughout the field study.

23. Related protocols:

n/a

24. Cost Estimate for Each Fiscal Year:

	FY 2008	FY 2009	FY 2010	FY 2011
USDA/NWRC:				
A. Salaries and Benefits	\$15,200	\$16,720	\$18,400	\$20,240
B. Facilities (in addition to existing facility or space costs)	0	0	0	0
C. Equipment	0	0	0	0
D. Supplies	\$4,000	\$1,500	\$1,500	\$1,500
E. Operating Costs (e.g., travel, misc. services, etc.)	\$500	\$500	\$500	\$500
TOTAL	\$19,700	\$18,720	\$20,400	\$22,240

25. Staff qualifications:

All study participants have documentation on file that verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study-specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study. All capture and immobilization operations during the proposed field study will be supervised by Margaret A. Wild, a wildlife veterinarian with the NPS who has had extensive experience in the capture and immobilization of ungulates at RMNP.

Study participants will include the following personnel: Dan L. Baker, Margaret A. Wild, Jenny G. Powers, Scott E. Ratchford, Bridget A. Schuler, Vicki Jameson, Amy L. Graham, Alicia M. Neiry, James P. Gionfriddo, and Kenneth A. Crane.

26. Archiving:

For the contraception subpart of the study plan (Appendix 29.1), all raw data, documentation, records, protocols, correspondence, and other documents relating to interpretation and evaluation of data, and the final report to the EPA will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado. Blood and tissue samples will be retained in the archives of the National Park Service.

27. Protocol Amendments:

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the study director, research program manager and sponsors. Amendments will be distributed to study participants as appropriate.

28. References:

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29. Appendices:

- 29.1 Study Plan: Evaluation of Methods for Managing Elk Population Health and Abundance in Rocky Mountain National Park, Colorado
- 29.2 Animal Use Appendix
- 29.3 Test, Control and Reference Materials/Device Use Appendix
- 29.4 Classification of Overall Body Condition of Captured Elk

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Signature Page:

James P. Gianfido
Study Director

1-2-2008
Date

Margaret H. H. H.
Study Sponsor (USDI/NPS)

2 Jan 2008
Date

Concur:

Kathleen A. Fagundes
Research Program Manager, NWRC

1-2-2008
Date

Approved:

Richard Bruggen
Director, NWRC

1/2/2008
Date

Processing:

QAU Received: 1/2/08 *L. Greiner* QAU Processed: 1/2/08 *L. Greiner*

IACUC (see IACUC approval date in Animal Use Appendix)

Appendix 29.2

STUDY PLAN

Evaluation of Methods for Managing Elk Population Health and
Abundance in Rocky Mountain National Park, Colorado

Principle Investigators:

Dan L. Baker, Department of Biomedical Sciences, Colorado State University

Margaret A. Wild, Biological Resource Management Division, National Park Service

Jenny G. Powers, Biological Resource Management Division, National Park Service

Co-Investigators:

Lowell A. Miller, National Wildlife Research Center, USDA/APHIS

Kathy A. Fagerstone, National Wildlife Research Center, USDA/APHIS

Mark S. Graham, Biological Resource Management Division, National Park Service

Therese L. Johnson, Rocky Mountain National Park, National Park Service

Mary Kay Watry, Rocky Mountain National Park, National Park Service

Terry R. Spraker, Veterinary Diagnostic Laboratory, Colorado State University

Katherine I. O'Rourke, Animal Disease Research Unit, USDA/ARS

Michael W. Miller, Wildlife Research Center, Colorado Division of Wildlife

Mary M. Conner, Department of Forest, Range, and Wildlife Sciences, Utah State
University

Terry M. Nett, Department of Biomedical Sciences, Colorado State University

[This study plan has been reviewed and approved by NPS and CSU, including the CSU IACUC.]

I. INTRODUCTION

Unregulated concentrations of elk (*Cervus elaphus*) have become a significant problem for resource managers in Rocky Mountain National Park (RMNP), Colorado. In particular, overabundant populations are severely altering native plant communities and potentially limiting the abundance and diversity of other wildlife species. Moreover, high densities of elk may serve as reservoirs for transmission of infectious diseases such as chronic wasting disease (CWD). In response to these concerns, park managers are considering alternative management strategies for reducing the size of the resident elk population.

One potential strategy is controlling the reproduction of female elk. Fertility control has been widely advocated as a non-lethal method for managing the growth of wildlife populations when lethal methods are impractical or unacceptable. Extensive research has been directed toward the development of different contraceptive technologies (Fagerstone et al. 2002) and simulation models have been constructed that offer general insight into the effects of fertility control on free-ranging wild ungulate populations. Modeling results are in broad agreement that the greatest efficiency for population control is achieved by combining culling with contraception such that an initial reduction in animal numbers is maintained by treatment with a long-acting fertility control agent (Hobbs et al. 2000, Merrill et al. 2003).

Unfortunately, efforts to develop a safe and efficacious contraceptive for free-ranging wild ungulates have been largely unsuccessful. Practical application has been limited by treatment duration, undesirable behavioral side-effects, and concerns for non-target species.

Recently, however, scientists at the National Wildlife Research Center, USDA/APHIS, Fort Collins, Colorado have developed an immunocontraceptive vaccine that has the potential to overcome these deficiencies. This approach offers several advantages over existing methods of contraception. These include:

1. a single treatment can provide multiple years of infertility
2. treatments can be safely and effectively applied at different times of the year to females in different reproductive states (non-pregnant or pregnant)
3. the agent appears to be safe for treated elk with minimal short-term physiological effects
4. the small volume required for effective contraception facilitates administration by syringe dart
5. there are no known adverse effects on non-target animals or the environment
6. regulatory approval of the vaccine for use in wild ungulates is currently in progress
7. the vaccine is relatively inexpensive (\$25-50 per dose) and could become commercially available

Preliminary investigations evaluating GnRH-Blue Protein/AdjuVacTM vaccine (GonaConTM) in captive wild horses (Killian et al. 2006), bison (Miller et al. 2004), white-tailed deer (Gionfriddo 2006), and elk (Killian et al. unpublished data) are promising and multi-year infertility has been achieved in all of these species. Particularly relevant to this investigation are the ongoing research projects with captive elk at the Wyoming Game and Fish Department's Tom Thorne/Beth William's Wildlife Research Facility, Wheatland, Wyoming, and the Colorado Division of Wildlife's Foothill's Wildlife Research Facility, Fort Collins, Colorado. In the former study, annual contraceptive efficiency (2005-2007) for female elk treated prior to the

breeding season was 86%, 90%, and 100%, respectively (Killian et al. unpublished data). In the latter study, 90% of pregnant elk treated with GonaConTM were found to be infertile after the first breeding season following treatment. Furthermore, there were only nominal effects of the vaccine on social reproductive behaviors and no adverse effects on the developing fetus, neonatal survival, or calf growth rates (Powers et al. unpublished data).

These studies provide strong inference on the potential utility of GonaConTM as a contraceptive agent for controlling reproduction in female elk and other wild ungulate species. However, results from these studies were obtained from a limited number of captive animals of known fertility, and in excellent physiological condition. They offer only a preliminary step in the evaluation of this contraceptive agent. A test of its success with wild elk is needed to assess whether this technology is truly effective and without undesirable side-effects.

Chronic wasting disease is a unique transmissible spongiform encephalopathy that occurs in captive and free-ranging deer (*Odocoileus* spp.), elk, and moose (*Alces alces*) in North America (Williams and Young 1980, 1982, Miller et al. 2000, Baeten et al. 2007). It was first identified in elk in RMNP in 1981. Prevalence of CWD in elk and mule deer (*Odocoileus hemionus*) adjacent to the park is estimated to be 3% and 5%, respectively (unpublished data, Colorado Division of Wildlife). It is not clear how CWD will affect the long-term health and viability of local cervid populations nor is it known how the presence of the disease will affect public perception of park elk. Thus, knowledge leading to the development of a practical and reliable test for preclinical CWD under field conditions would assist wildlife managers in making decisions on the health of elk populations in RMNP. While antemortem tests for diagnosing CWD are available for use in mule deer and white-tailed deer (*Odocoileus virginianus*) (Wild et al. 2002, Wolfe et al. 2002, Wolfe et al. 2007) there is no reliable live-

animal test to identify elk that are subclinically affected with CWD. However, recent studies have reported two potential techniques that are worthy of further investigation. One method involves sampling the lymphoid tissue found in rectal mucosa (Spraker et al. 2006) and the other relies on a blood plasma test capable of detecting prions. The latter approach uses *in vitro* amplification (Am) combined with aggregation-specific (AS) fluorescent amplification catalyzed by a T7 RNA polymerase technique (FACTT) and has been used experimentally to identify prions in blood samples from mule deer infected with CWD (Chang et al. 2007). These antemortem tests for CWD require further evaluation but may provide methods to grossly estimate prevalence of the disease in elk in RMNP.

Development and progression of CWD has been shown to be related to PrP genotype in elk (O'Rourke et al. 1999, Hamir et al. 2006) white-tailed deer (Johnson et al. 2003, O'Rourke et al. 2004, Wolfe et al. 2007), and mule deer (Jewell et al. 2005, Wolfe et al. 2007) thus making it an important to consideration for evaluating the effects of CWD on a cervid population.

Thus, as part of the adaptive management approach, described in the Elk and Vegetation Management Plan/Environmental Impact Statement (EIS) under alternative 4, and in coordination with RMNP managers' plan to lethally remove elk, we propose to opportunistically evaluate methods for managing elk population health and abundance in RMNP. Our specific objectives are to:

1. determine the efficacy of GonaConTM as a long-term contraceptive agent in female elk and assess the durability of its effects over a 3-year period.
2. assess the physiological side-effects (if any) of GonaConTM treatments on female elk (i.e. survival, body condition, injection site reactions)

3. provide a gross estimate of CWD prevalence in selected subpopulations of female elk in RMNP
4. determine the effectiveness of CWD diagnosis by comparing rectal biopsy immunohistochemistry (IHC) and blood plasma Am-A-FACTT, to conventional tissue (obex, retropharyngeal lymph node, spleen) for evidence of abnormal prion protein.
5. evaluate the capability of the rectal biopsy and blood assay to diagnose CWD prior to the appearance of clinical signs of the disease.
6. investigate the relationship between PrP genotype and CWD in RMNP elk

In addition to the primary objectives, the study design developed here offers the opportunity to gather information on the following:

1. an initial assessment of the effects of culling on movement, distribution and habitat selection of park elk
2. estimates of population parameters (i.e. census, population growth rates, adult female elk survival)
3. provide estimates of contraception efficiency in elk in RMNP and use this data to revise fertility control simulation models (Hobbs and Bradford, unpublished data) to more precisely predict population responses

II. METHODS

A. Study Area. We will conduct this study on elk winter ranges in RMNP. Elk winter in the eastern portion of the park and adjacent Estes Valley, which includes the town of Estes Park, Colorado. Glacial moraines extending eastward from the Continental Divide separate the winter range into 4 parallel valleys: Beaver Meadows, Horseshoe Park, Moraine Park, and Hollowell

Park (Gysel 1959). Experimental animals will primarily be selected from subpopulations located in Moraine Park/Beaver Meadows and Horseshoe Park.

B. Experimental Procedures. During approximately 1 January to 15 February, 2008, up to 120 adult female elk will be immobilized on selected winter ranges in the park. We anticipate that approximately 25% of experimental elk will not be recovered as part of management culling operations. Factors affecting the loss of experimental elk are difficult to assess but may be due, in part, to radiocollar failure, disease, hunting loss outside the park, predators, and emigration from the park. Therefore, to meet a sampling rate of 30 elk/year for 3 years, a total of 120 elk will be initially captured, treated, sampled and radiocollared. This will be accomplished by driving the roads and locating groups of elk. Individual animals will be evaluated for clinical signs of CWD, then darted from a vehicle or stalked on foot. We will attempt to choose only female elk two or more years in age as experimental animals. Selected elk will be randomly assigned to either a treatment or control group. Treatment elk will receive an intramuscular injection in the hip, by hand-syringe, containing 2000 ug of GnRH-Blue Protein conjugate emulsified in AdjuVacTM adjuvant (GonaConTM). Control females will be injected with saline solution.

Field anesthesia will be accomplished using a mixture of 2.7 mg of carfentanil (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and 10 mg xylazine (Tranquived, Vedco, St. Joseph, Missouri, USA) delivered in a barbed, 1 or 2 cc self-injecting dart, fired from a CO₂ rifle (Dan-InjectTM, Dan-Inject of North America, Fort Collins, Colorado, USA). Capture locations will be recorded utilizing handheld GPS units. Immediately following immobilization, we will blindfold the elk, monitor vital signs, estimate age by tooth replacement and wear (Quimby and Gaab 1957), determine pregnancy status, collect blood (30 ml), biopsy the rectal mucosa for

CWD testing (Spraker et al. 2006), and assess general body condition (Riney 1960). We will confirm pregnancy using 2 independent methods: rectal palpation of the fetus, fetal membranes, or uterine cotyledons (Greer and Hawkins 1967) and serum analysis for pregnancy-specific protein B (BioTracking, Moscow, Idaho, USA) (Noyes et al. 1997).

We will fit all experimental elk with VHF radiocollars (Advanced Telemetry Systems, Isanti, Minnesota, USA). Radiocollars will be sheathed by a plastic identification sleeve marked with a unique alpha-numeric code of 76 mm-high black characters on a colored background (white for control; yellow for treatment) (Freddy 1993). Radiocollars will be marked with the warning "Do Not Consume" and agency contact information. We will reverse the effects of the immobilization drugs with 300 mg naltrexone (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and 30 mg yohimbine (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). To minimize the possibility of infection associated with immobilization, each elk will receive a subcutaneous dose (6,000,000 IU) of long-acting penicillin (Durapen, Vedco, Incorporated, St. Joseph, Missouri, USA).

C. Measurements.

1) **Reproductive rates.** We will determine the effects of GonaConTM on elk pregnancy rates during 1 January and 15 February, 2009-2011. In coordination with RMNP managers' lethal removal operations, approximately 30 experimental elk (15 controls; 15 treatment) will be killed each year by certified sharpshooters. We will conduct postmortem examination of the carcass, confirm pregnancy by inspection of the reproductive tract for presence or absence of a fetus, preserve the ovaries, uterus, pituitary and hypothalamus for later analysis, and collect samples for CWD assays.

2) **Body condition.** We will assess the effects of GonaCon™ on elk body condition by comparing differences in body fat of treated and control elk collected during lethal removal operations. We will use a body condition scoring system developed by Kistner (1980) which estimates (1) fat in selected indicator sites (cardiac, omental, perirenal, and subcutaneous areas, and (2) the condition of skeletal muscle mass. For dead animal body condition evaluation, these indices have been shown to provide a practical and acceptable field technique for predicting body composition in elk (Cook et al. 2001a, b).

3) **Injection site reactions.** At the time of vaccine application (2008), we will use a permanent marking method to facilitate relocation of the injection site at necropsy. At necropsy, we will determine the number of treated and control elk with abscesses, granulomas, and scarring by removing the section of muscle where the injection was given, and examining it for gross and histopathological lesions.

4) **CWD antemortem test and prevalence rates.** We will evaluate rectal biopsy and blood assays as live-animal field tests for CWD by sampling experimental elk during 2008-2011. Rectal biopsy sampling will require removing two small (1.5 x 1 cm) strips of mucosal tissue from the wall of the rectum approximately 1 cm anterior to the mucocutaneous junction of the anus (Spraker et al. 2006). Tissue samples will be collected at the 10 and 2 o'clock positions. Samples will be preserved in 10% neutral buffered formalin and processed for CWD analysis using immunohistochemical techniques. We will make an initial gross estimate of CWD prevalence by dividing the total number of CWD positive samples by the sample population obtained in 2008. Any elk testing positive on either rectal biopsy or blood assays will be lethally removed from the population and transported to Colorado State University for necropsy. We will compare rectal tissue with brainstem and/or retropharyngeal lymph nodes via IHC (Williams et

al. 2002) to evaluate diagnostic efficacy of rectal tissues. Additionally, we will evaluate the rectum to determine if long-term changes are visible either grossly or on histopathological examination. If possible, plasma will be collected at the time of death to compare conventional IHC with the blood assay.

5) **Relationship of clinical signs to rectal biopsy.** Prior to anesthesia (2008) or culling (2009-2011), each animal will be observed for clinical signs of CWD. Clinical signs include: poor body condition, lowered head/ears, salivation, abnormal behavior, head tremors, and remaining near water sources for extended periods. A subjective scoring system for clinical signs will be developed or modified from those already in use (M. Miller personal communication). We will compare CWD test results with results from field observations.

6) **Survival.** To determine survival and general movement patterns of elk, we will periodically (approximately 1-week intervals) locate as many experimental animals as possible by driving the roads and using radiotelemetry. If a mortality signal is observed during routine monitoring activities, we will attempt to locate and remove the carcass for full diagnostic evaluation at Colorado State University's Veterinary Diagnostic Laboratory, Fort Collins, Colorado. Survival rates and cause of death will be compared between treatment and control groups.

7) **PrP Genotyping.** We will determine the genotype of experimental elk according to procedures described by O'Rourke et al. (2004). Blood samples will be sent to K. O'Rourke (USDA/ARS) for PrP genotyping and genetic analysis. Results from this laboratory will be analyzed to evaluate the relationship between PrP genotyping and spatial and temporal changes in CWD prevalence among female elk from selected subpopulations in park.

D. Statistical analysis. We performed a power-based sample size determination for a 1-sided Fisher's exact test (Krishnamoorthy and Thompson 2002, Kang and Kim 2004) using a software program (NCSS Pass 2000) to determine the sample size required to detect differences in pregnancy rates between treated and control elk. For this analysis, we assumed a 90% infertility rate the first year following treatment, and 85% for year 2, and 80% for year 3, 80% recapture success for all experimental elk, and 100% accuracy of detecting pregnancy at necropsy. For control elk, we used an 80% pregnancy rate that was determined in a previous study in RMNP (Conner et al. 2007, in press). Our null hypothesis is that the minimum difference in pregnancy rates will be 0.80, with pregnancy rates of untreated elk greater than treated elk. Based on this analysis, 30 female elk (equally divided between 2 groups) would need to be collected each year to provide adequate power ($\geq 80\%$) to detect expected differences in pregnancy rates. We anticipate that 30 of these elk will be available annually for collection during ongoing management culling activities.

E. Schedule.

Date	Activity
June 2007	Prepare study proposal and submit to Environmental Protection Agency for experimental animal use permit
January - February 2008	Capture, treat, and sample 120 elk in RMNP
January – March 2008	Lethally remove elk that have tested positive for CWD
January 2008 - March 2011	Monitor survival of experimental elk; locate and remove carcasses for diagnostic examination
January 2009 – March 2011	Lethally remove and necropsy 30 experimental elk each year (January-March) for determination of pregnancy rates, side-effects of treatment (if any), and CWD evaluation.
December 2011	Analyze data and prepare final report

III. LITERATURE CITED

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Appendix 29.2

Animal Use Appendix

[Protocols for this field study were reviewed and approved by the CSU IACUC. The information presented below is provided as a quality assurance/quality control check for compliance of those protocols.]

Animal use is defined as the use of any vertebrate, including manipulating the behavior of wild animals in their natural habitat.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal description:

- 1) Species: elk (*Cervus elaphus*)
- 2) Strain and substrain (if applicable): n/a
- 3) Number and Sex: 120 females
- 4) Body weight range: 225-275 kg
- 5) Age: adult (≥ 2 years old)

B. Rationale for involving animals, for appropriateness of species, and for numbers: *(For example, is there a model or surrogate species that might satisfy the needs of the study.)*

- 1) Rationale for involving animals: Non-lethal techniques such as contraception are needed to manage cervid populations in areas where traditional methods of management (e.g., sport hunting) are prohibited or non-feasible. At RMNP, overabundant elk are causing severe ecological damage. A contraceptive agent that is likely to reduce reproduction in elk may be useful in the future as a management tool at RMNP and in other locations where overabundant elk cause problems. The most appropriate way to evaluate the safety and efficacy of wildlife contraceptive agents such as GonaCon™ vaccine is by testing them in populations of free-ranging animals in natural environments.
- 2) Rationale for appropriateness: Elk are responsible for many ecological problems at RMNP, and it is their population that must be managed and reduced to restore the ecological integrity of native plant and animal communities. There is no suitable alternative to using elk in this field study.
- 3) Rationale for numbers (include calculations as appropriate): To demonstrate unequivocally that the contraceptive agent (GonaCon™) is responsible for any differences that are detected in reproductive success between vaccinated and control elk, it will be necessary to capture, mark, and monitor the reproductive success of a sufficient number of elk. Two previous field studies of the efficacy of GonaCon™ immunocontraceptive

vaccine in free-ranging white-tailed deer involved the use of 28 vaccinated does (per study), in Maryland and New Jersey. Mortality of vaccinated deer during these two-year field studies substantially reduced the effective sample sizes. The proposed field study will be located in a very different geographic region of the United States, and will involve the contraception of a different cervid (genus and) species. The final, effective annual sample sizes for vaccinated elk at RMNP must be adequate to demonstrate a treatment effect of vaccination, if one exists. The 120 elk that will be involved in this study will include 60 animals that will be captured and treated with GonaCon™ vaccine during 2008 and 60 control animals that will be captured and given injections of a sham material (sterile saline solution) during 2008. Power analysis conducted by the NPS determined that sample sizes of 60 treated and 60 control elk would be needed to clearly demonstrate treatment effects (statistically) if they occur in this study.

C. Source:

Elk will be captured on their winter range within the eastern portions of Rocky Mountain National Park, near the town of Estes Park in north-central Colorado.

D. Method of identification of animals:

All captured elk used in the study will be equipped with plastic, uniquely-numbered identification sleeves (attached to the radio telemetry collars) that will enable field workers to identify individual animals from a distance by using binoculars or spotting telescopes. In addition, experimental elk will be fitted with radio telemetry collars and transmitters. Each animal's transmitter will emit a radio signal that has a unique radio frequency, and will be equipped with a mortality sensor that will be activated after 4 continuous hours of non-movement (of the elk and collar). Although experimental elk will not be monitored via radio telemetry as part of this study, the presence of the transmitters on the elk will permit the NPS, if personnel are available, to monitor the survival of the animals. If the NPS documents mortality of experimental elk, the NWRC will be notified of such mortality.

E. Trapping/Collecting:

Elk will be captured during January-February 2008 at several specific locations where they congregate near paved roads in relatively open habitats at RMNP. Elk will be captured with tranquilizer darts which will be fired from specially-designed CO₂ rifles (Dan-Inject of North America, Fort Collins, Colorado, USA). Each (1 or 2 cc) tranquilizer dart will contain a mixture of 2.7 mg of carfentanil (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and 10 mg of xylazine (Tranquived, Vedco, St. Joseph, Missouri, USA). Anesthesia of darted elk will be reversed with an injection of a mixture of 300 ml of naltrexone ((1/4 intravenous, 3/4 subcutaneous; Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and 30 mg of yohimbine (intravenous; Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). Additional

details regarding capture and immobilization procedures are provided on pages 5-9 of this protocol.

F. Transport:

Elk will not be transported during this field study.

G. Handling/restraint:

Elk will be chemically immobilized during the capture process. Additional details regarding the chemical immobilization of study elk are provided on pages 5-9 of this protocol.

H. Quarantine:

Elk will not be quarantined during this field study.

I. Housing/maintenance:

Elk will not be taken into captivity or housed during this field study.

J. Disposition of animals:

During January and February of each year during the period 2009-2011, 40 experimental elk (20 GonaCon™-vaccinated and 20 control animals) will be shot and killed by trained sharpshooters. These animals are to be killed as part of the elk population reduction operation at RMNP. The 40 elk will receive careful postmortem examinations, and blood and tissue samples will be collected for subsequent laboratory evaluation.

If non-scheduled mortality of experimental elk occurs and if carcasses can be reached before they are consumed by predators or scavengers, the carcasses will be collected and taken to the Colorado State University Veterinary Diagnostic Laboratory for necropsy, tissue sampling, and disposal. If intact carcasses are found in locations that are long distances from roads, then tissue samples will be collected in the field and the remaining portions of the carcasses will be left in the field for consumption by scavengers.

The long-term goal of the management plan for elk at RMNP is the reduction of the population to about 1,600 to 2,100 animals over a 20-year period. The use of lethal control to reduce the elk population provides an opportunity to test GonaCon™ Immunocontraceptive Vaccine in a new cervid species under ambient field conditions.

K. Duplication of prior studies:

The proposed field study will evaluate GonaCon™'s efficacy in a cervid (genus and) species in which it has not been field-tested, and it will evaluate GonaCon™ in a geographic region of the United States in which it has not been field-tested.

L. Pain or distress:**Consultation with Attending Veterinarian:**

Consult with the Attending Veterinarian to determine if any portion of the study will cause more than momentary or slight pain or distress, and to address any animal care and use issues prior to procedures being initiated. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Dr. Pauline Nol

Date of Consultation: December 4, 2007

Is this study expected to cause more than momentary or slight pain or distress?

Yes: No: X

If the Attending Veterinarian determines procedures may cause more than momentary or slight pain or distress, continue with the following items. If not, indicate as N/A.

1) Alternative procedures:

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

N/A

2) Sedatives, analgesics, or anesthetics:

a) Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

- i) Each tranquilizer dart will contain a mixture of 2.7 mg of carfentanil and 10 mg of xylazine. Anesthesia of darted elk will be reversed with injections of 300 mg of naltrexone (¼ intravenous and ¾ subcutaneous) and 30 mg of yohimbine (intravenous).
- ii) If an elk is injured during the capture operation, the supervising veterinarian (Dr. Wild) will examine the animal to determine if it is in good enough condition to be released. If it is determined that the severity of the injury precludes release, then the animal will be euthanized with a gunshot to the head at close range or with an

injection of an overdose of barbiturates. If injuries are relatively minor (e.g., minor lacerations and abrasions), then the animal may be treated on site with methods ranging from administration of an injectable antibiotic and Clostridial vaccine to disinfection and suturing of a wound. If a sedative is required (as when a laceration is sutured), use of the following drugs would be considered:

- i. xylazine (1.5 - 2.0 mg/kg, intranasally via rubber pediatric feeding tube); reverse with tolazoline (2.0 - 4.0 mg/kg, half intravenously and half intramuscularly)
- ii. ketamine (2.5 mg/kg) and medetomidine (0.1 mg/kg); reverse with atipamezole (0.5 mg/kg, half intravenously and half intramuscularly).

The use of these drugs would be at the discretion of the supervising veterinarian and would depend on many factors, including animal stress levels, vital signs, ambient air temperature, location, age, and reproductive condition.

- b) If sedatives, analgesics, anesthetics are needed to minimize or alleviate discomfort, distress or pain but will be withheld, provide a written justification (referred to as the "Column E" justification") or otherwise indicate "no Column E justification required".

No Column E justification required.

3) Surgery:

Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

N/A

M. Euthanasia:

Each study elk that becomes critically ill or injured during the capture phase of the study will be euthanized by a gunshot to the head (at close range) or by the injection of an overdose of barbiturates.

N. IACUC approval:

Date of IACUC Approval Letter: December 28, 2007

Appendix 29.3 Test, Control and Reference Material/Devices Formulation and Use Appendix

(NWRC Required Form)

[The test material (GonaCon™ Immunocontraceptive Vaccine) will be transferred to NPS/CSU under a Material Transfer Agreement that will specify the conditions of use and reporting requirements.]

A. Describe the test material:

As appropriate, for each material provide the chemical, bait or device

- 1) name or code: GonaCon™ immunocontraceptive vaccine
 - a) concentration: 1000 micrograms/ml; purity: n/a
 - b) source: manufactured by NWRC
 - c) batch number: to be determined

B. Describe any control or reference materials/devices:

No control or reference materials/devices are to be used.

C. Carriers, mixtures and material preparation:

Vaccine Ingredients:

Each 1.0 ml dose of the GonaCon™ formulation contains the following ingredients:

GnRH/KLH Conjugate (1000 µg)

Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
<i>Concholepas concholepas</i> hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml

AdjuVac™ adjuvant

<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

D. Route of administration:

GonaCon™ will be administered to each elk via an intramuscular injection in the hindquarters because this route of administration yields the longest contraception duration.

E. Dosage:

A single 2-ml dose of GonaCon™ Immunocontraceptive Vaccine will be given to each elk.

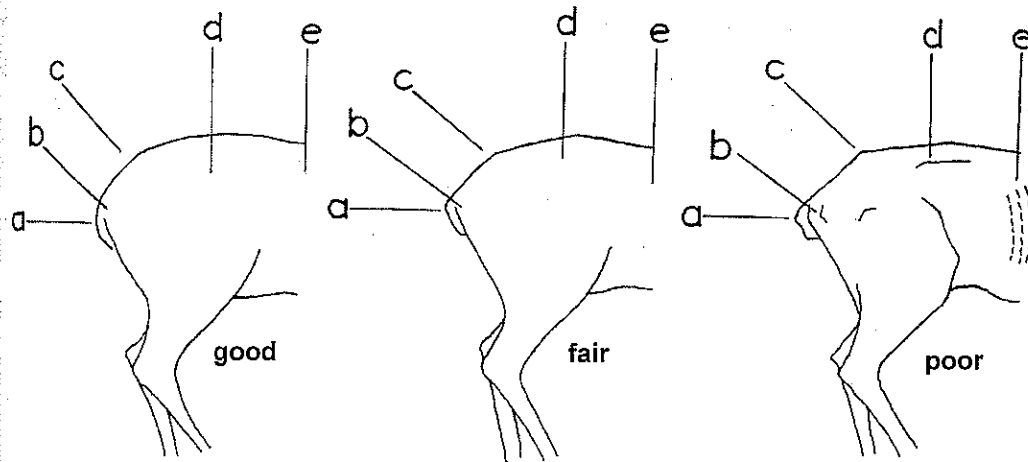
F. Test, control, and reference substance accountability:

Storage and usage of GonaCon™ immunocontraceptive vaccine will be tracked via chain of custody forms. Unused vaccine will be returned to Dr. Lowell A. Miller at NWRC and then disposed of by incineration.

G. Material verification:

No material verification will be made because no analytical method capable of providing such verification exists. The vaccine manufacturing process will follow an established SOP and will be inspected by NWRC's quality assurance officer.

Appendix 29.4 Classification of Overall Body Condition of Captured Elk



After Riney (1960)

"As the fat reserves of the animal diminish, the tail (*a* [in above figure]) appears more angular, the outline of a point of the pelvic girdle (*b*) can be seen, a distinct angle appears at the point indicated by (*c*), the lateral processes of the backbone vertebrae (*d*) can be seen as a faint line, and outlines of the ribs (*e*) are visible. If there are no angles corresponding to points *a* and *c* on the diagram, the deer are classed as in good condition ([above], left). If any one of the points indicated at *b*, *d*, or *e* can be observed, the deer is classed as in poor condition ([above], right). [The image in the middle, above] represents the appearance of individuals that are not clearly in good condition or poor condition." (Riney 1960:93).

Standard Operating Procedures (SOPs) are internal working documents of the National Wildlife Research Center. These documents are confidential to NWRC personnel and should not be distributed.

Standard Operating Procedures

revised 11/9/10

PDF files for most of these SOPs can be accessed on the NWRC Intranet

AD - ADMINISTRATIVE

AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 003.03	Research Protocols
AD 004.01	Archiving Studies
AD 005.02	Archive Maintenance and Access
AD 006.00	TERMINATED
AD 007.02	Final Reports
AD 008.01	Personnel Qualification Records
AD 009.01	Institutional Animal Care and Use Committee
AD 010.01	Standard Format for Data Submissions to EPA
AD 011.02	Data Recording and Error Correction
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 013.00	Designation of Study Director
AD 014.00	Adverse Incident Reporting FIFRA 6(a)(2) - A guidance document for study directors
AD 015.01	Institutional Biosafety Committee
AD 016.01	Approval and training for BSL-3 workers and visitors
AD 017.01	NWRC Code of Scientific Ethics
AD 018.00	NWRC BSL-3 Oversight Committee
AD 019.01	Handling Allegations of Research Misconduct
AD 020.03	Establishing Authorship and Review of NWRC Manuscripts
AD 021.00	Labeling requirements
AD 022.00	Laboratory Housekeeping
AD 023.00	Assigning Chemical Expiration Dates

AC - ANIMAL CARE

AC 001.00	Cervical dislocation as a humane means of killing captive birds
AC 003.00	Collecting & Processing blood samples from medium-sized mammals
AC 004.00	Handling and restraint of medium-sized mammals
AC 005.00	Capturing, handling, and caring for mongooses (<i>Herpestes auro-punctatus</i>)
AC/CO 001.01	Ground Squirrel Maintenance ARB (shoebox cages)
AC/CO 002.00	Animal Handling to Maintain Secure Identification
AC/CO 003.01	Quail Maintenance (caged in rabbit racks) ARB
AC/CO 004.00	Peromyscus Maintenance and Breeding
AC/CO 005.01	Mouse Maintenance (shoebox cages) ARB
AC/CO 006.01	Pocket Gopher Maintenance ARB (shoebox cages)
AC/CO 007.01	Ferret Maintenance ARB (stainless steel rabbit racks)
AC/CO 008.00	Euthanasia with CO ₂
AC/CO 009.00	Animal Care (Biosafety Level 2)
AC/CO 010.00	Bird Maintenance (Free Flight)
AC/CO 011.02	Rat Maintenance ARB (shoebox cages)
AC/CO 012.00	Starling Maintenance (caged)
AC/CO 013.02	Crow maintenance OARF (free flight)
AC/CO 014.01	Brown Tree Snake Maintenance ARB (caged)
AC/CO 015.02	Rabbit Maintenance ARB
AC/CO 016.00	Animal Quarantine Procedures at Fort Collins
AC/CO 017.02	Animal Food Storage at the ARB and ISRB
AC/CO 018.01	African House Snake Maintenance ARB
AC/CO 019.00	Vole Maintenance (S. N. E.)
AC/CO 020.00	Incinerator use at the Animal Research Building
AC/CO 021.00	Dove Maintenance (Free Flight, S.N.E.)
AC/CO 022.00	Coyote maintenance OARF
AC/CO 023.00	Necropsy room procedures
AC/CO 024.00	Mouse-scrapie model: Handling procedures for mice, mouse cages and animal waste
AC/CO 025.01	Mouse-scrapie model: Procedures for scrapie brain homogenate and mouse necropsies
AC/CO 026.00	Blackbird maintenance OARF (caged)
AC/CO 027.00	Raccoon Maintenance OARF
AC/CO 028.00	Waterfowl maintenance OARF
AC/CO 029.00	Rat maintenance ARB (suspended cages)
AC/CO 030.00	Pileated Woodpecker Maintenance OARF (free flight)
AC/CO 031.00	Pigeon Maintenance OARF (caged and free flight)
AC/CO 032.00	Blackbird Maintenance (caged) in the ARB
AC/CO 033.00	Skunk Maintenance for OARF
AC/CO 034.00	Quail maintenance OARF (indoor aviary)
AC/CO 035.00	Blackbird maintenance OARF (free flight)

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AC/CO 036.00	Fox Squirrel Maintenance OARF (stainless steel rabbit racks)
AC/CO 037.00	Animal Care - Biosafety Level 3
AC/CO 038.00	Disposal of animal carcasses in the BSL-3 facility
AC/CO 039.00	BSL-3 animal room entry and exit
AC/CO 040.00	Care and maintenance of swallow bug (<i>Oeciacus vicarius</i>) colony
AC/CO 041.00	Robin maintenance (individually caged) in the Outdoor Animal Research Facility (OARF)
AC/CO 042.00	Incubation and hatching of chicken eggs
AC/CO 043.00	Short term Prairie dog maintenance (individually caged) in the Outdoor Animal Research Facility (OARF)
AC/CO 044.00	Crow maintenance OARF (caged)
AC/CO 045.00	Prairie dog maintenance in the Simulated Natural Environment (SNE) Rooms in the Animal Research Building (ARB)
AC/CO 046.00	Prairie dog maintenance ARB (stainless steel rabbit racks)
AC/CO 047.00	Group Housed Quail Maintenance in the Outdoor Animal Research Facility (OARF) Rodent Buildings (bldg. 22, 23, and 24)
AC/CO 048.00	Ferret Maintenance Indoor Group Housed
AC/CO 049.00	Gull maintenance for Outdoor Animal Research Facility (OARF)
AC/CO 050.00	Animal Care Record Maintenance
AC/CO 051.00	Gambian Rat Maintenance ISRB (Stainless steel rabbit racks)
AC/CO 052.00	Monk Parakeet Maintenance ISRB (caged in SNE)
AC/CO 053.00	Coconut Crab Maintenance (plastic or vinyl containers)
AC/CO 054.00	Nile Monitor Maintenance ISRB
AC/CO 055.00	Big Brown Bat Maintenance (caged in ARB)
AC/CO 056.00	Bullfrog maintenance (group-housed in large plastic tank in Invasive Species Research Building)
AC/CO 057.00	Maintenance and breeding of specialized mouse sources for use in research
AC/CO 058.00	Bobcat maintenance for OARF
AC/FL 001.01	Raccoon (<i>Procyon lotor</i>)
AC/FL 002.01	Opossum (<i>Didelphis virginiana</i>)
AC/FL 003.01	Monk Parakeets (<i>Myiopsitta monachus</i>)
AC/FL 004.01	House Sparrow (<i>Passer domesticus</i>)
AC/FL 005.00	Necropsy of birds
AC/FL 006.01	House Finches
AC/FL 007.01	Animal Care - General
AC/FL 008.00	Handling of animals found mor bund or dead during study
AC/FL 009.01	Blackbirds and Starlings
AC/FL 010.00	Transfer, proper placement, and identification of animals
AC/FL 011.00	Animal care - mealworms (<i>Tenebrio molitor</i>)
AC/FL 012.00	Black vulture (<i>Coragyps atratus</i>) Turkey Vulture (<i>Cathartes aura</i>)
AC/HI 001.00	Housing procedures for francolins and other gallinaceous birds at the Hilo Field Station
AC/HI 002.00	Euthanasia with carbon dioxide (CO ₂) gas at the Hawaii Field Station
AC/HI 003.00	Brief guidelines for the necropsy of rodents and small mammals
AC/HI 004.00	Storage of rodent feed at the Hawaii Field Station
AC/HI 005	TERMINATED – replaced by FP/HI 002
AC/HI 006.00	Live trapping, handling, processing, and care of mice (<i>Mus musculus</i>) at the Hawaii Field Station
AC/HI 007.00	Live trapping, handling, processing, and care of rats (<i>Rattus spp.</i>) at the Hawaii Field Station
AC/HI 008.00	Non-native parakeets (<i>Aratinga</i> , <i>Cyanoliseus</i> , <i>Psittacula spp.</i>) and parrots (<i>Amazona</i> , <i>Poicephalus</i> , <i>Psittacus spp.</i>)
AC/HI 009.00	Live capture, handling, processing, and care of tree frogs (<i>Eleutherodactylus spp.</i>)
AC/MS 001.01	Maintenance of fish at the Mississippi Research Station
AC/MS 002.00	Euthanasia of birds and fish at the NWRC Starkville Field Station
AC/MS 003.00	Maintenance of wading birds, cormorants, and pelicans at the Mississippi Field Station
AC/MS 004.00	Necropsy procedures for fish-eating birds
AC/ND 001.00	Care of red-winged blackbirds, yellow-headed blackbirds, common grackles, brown-headed cowbirds, and European starlings
AC/OH 001.00	Small bird care and maintenance
AC/OH 002.00	Capture of blackbirds and starlings using decoy and nest traps
AC/UT 001.00	Daily coyote check and care for the Millville Predator Research Facility
AC/UT 002.00	Procedures for euthanizing coyotes (<i>Canis latrans</i>)
AC/WA 001.00	Animal feeds and storage of feed and medication
AC/WA 002.00	Ground transport of mountain beaver (<i>Aplodontia rufa</i>)
AC/WA 003.00	Care of small mammals (rodentia)
AC/WA 004.01	Care of pocket gophers (<i>Thomomys mazama</i>) at the Olympia Field Station
AC/WA 005.00	Care of porcupine (<i>Erethizon dorsatum</i>)
AC/WA 006.00	Care of captive stream beaver (<i>Castor canadensis</i>)
AC/WA 007.00	Care of captive deer (<i>Odocoileus spp.</i>)
AC/WA 008.01	Care of individually housed captive mountain beaver (<i>Aplodontia rufa</i>) at the Olympia Field Station
AC/WA 009.00	Care of group housed captive mountain beaver (<i>Aplodontia rufa</i>)
AC/WA 010.00	Air transport of mountain beaver (<i>Aplodontia rufa</i>)
AC/WS 001.00	Care of captive nutria (<i>Myocaster coypus</i>) Blackwater National Wildlife Refuge-Cambridge, MD

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ANIMAL CARE -- WRC SOPs that are current

WRC-11R1	Aerial capture techniques for live capture of coyotes
WRC-12R3	Collection, handling, and storage of blood from live coyotes
WRC-14R1	Capture and handling of coyotes for research purposes
WRC-25R1	Routine maintenance and care of sheep and goats at the Millville (UT) Research Facility
WRC-28R2	Administering chemical doses to coyotes (<i>Canis latrans</i>)
WRC-50R1	Handling procedures for fox
WRC-265R3	Oral gavage of bird species
WRC-268R1	Collection, handling and storage of blood from live sheep/goats
WRC-288R3	Waterfowl maintenance (Ohio field station)
WRC-289R3	Gull maintenance (Ohio field station)
WRC-296R1	Pigeon maintenance (Ohio field station)
WRC-329R3	Animal care - frugivorous birds
WRC-359R3	Routine veterinary medical procedures for coyotes
WRC-360R1	Procedures for receipt of coyotes
WRC-361	Coyote handling and transport at the Millville Pred. Res. Fac.
WRC-363	Procedures for rearing and handling coyote pups
WRC-375R1	Procedure for euthanasia of double-crested cormorants at the Mississippi Research Station
WRC-404	Transport of Canada geese (ground)
WRC-488	Capture, handling, and care of mongooses (<i>H. auropunctatus</i>)
WRC-559	Cage breeding of the Norway rat

BT - BIOTECHNOLOGY

BT 001.00	ELISA procedure for assessing immune responses
BT 002.00	Peroral immunization with attenuated bacteria
BT 003.00	Hapten/carrier conjugation
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
BT 005.00	Heat inactivation of blood samples
BT 006.00	General E. coli assay
BT 007.00	Biochemical confirmation tests used for survey of E. coli
BT 008.00	Collection and preservation of avian tissue for histopathological and immunohistochemical examination
BT 009.00	Collection & processing of blood spot samples from small birds
BT 010.00	Premier Enterohemorrhagic E. coli enzyme linked immunoassay kit (EIA)
BT 011.00	TaqMan reverse transcriptase-polymerase chain reaction assay for rapid detection of W. Nile virus in birds
BT 012.00	Salmonella spp. assay for avian and environmental samples
BT 013.01	Inventory and storage procedures for BSL2 agents and diagnostic samples
BT 014.00	Blocking ELISA screening procedure to test avian sera for W. Nile antibodies (IgM and IgG)
BT 015.00	Indirect ELISA procedures for West Nile Virus IgG Antibody Detection
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine using the Microfluidics M110L high pressure homogenization instrument and/or the manual hand method
BT 017	pending
BT 018.00	Virus isolation by plaque assay
BT 019.00	Plaque Reduction Neutralization Test (PRNT)
BT 020.00	Avian Influenza (AI) Agar Gel Immunodiffusion Test (AGID)
BT 021.05	Reverse Transcription and Real-Time Polymerase Chain Reaction (RT-PCR) for Rapid Detection of Avian Influenza in Environmental Samples
BT 022.00	Preparation of Erythrocytes for Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Assays
BT 023.00	Hemagglutination (HA) and Hemagglutination-Inhibition (HI) tests for influenza A virus
BT 024.00	Avian influenza virus isolation in embryonating chicken eggs
BT 025.00	Use of Radioimmunoassay (RIA) to assess reproductive hormone concentrations in plasma or serum
BT 026.00	Purification of antibodies from plasma or serum using the Pierce NAb Spin Kit
BT 027.00	<i>Histoplasma capsulatum</i> assay for fecal and soil samples
BT 028.00	Terminated – replaced by BT 016.02
BT 029.00	Extraction of Avian influenza and/or Newcastle Disease Viral RNA using the KingFisher 96
BT 030.00	Manufacture of 0.23% DiazaCon (w/w) rolled oat bait for prairie dogs
BT 031.00	Extraction, Amplification, and Visualization of <i>Mycobacterium bovis</i> DNA in Feces
BT 032.01	DNA extraction from dried blood spots using the DNeasy Blood and Tissue kit
BT 033.00	DNA extraction from hair using the QIAamp DNA Micro kit
BT 034.00	DNA extraction from swabs using the QIAamp DNA Micro kit
BT 035.01	DNA extraction from tissue using the DNeasy Blood and Tissue kit
BT 036.00	DNA extraction from whole blood using the DNeasy Blood and Tissue kit
BT 037.00	Laboratory handling of live rabies virus (Rabies lab specific procedures)
BT 038.00	General PCR Procedure
BT 039.00	Fragment Analysis on Applied Biosystems Genetic Analyzer
BT 040.00	Cycle Sequence Reaction
BT 041.00	Visualizing DNA or PCR Product with Electrophoresis
BT 042.00	ExoSAP-IT PCR Product Clean-up
BT 043.00	Sephadex Cycle Sequence Clean-up
BT 044.00	Use of Radioimmunoassay (RIA) to Assess Prolactin Concentrations in Plasma or Serum
BT 045.00	Sequence Analysis on Applied Biosystems Genetic Analyzer
BT 046.00	Purification of DNA using GENECLEAN III Kit
BT 047.00	DNA extraction from fecal samples using the QIAamp DNA Stool Mini Kit
BT 048.00	Serial Protein Misfolding Cyclic Amplification (sPMCA) and preparation of required brain homogenate substrate

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BT 049.00	Western Blot Analysis of CWD-associated samples
BT 050.00	Intercerebral inoculation of control and prion-containing material
BT 051.00	Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) for the detection of West Nile Virus
BT 052.00	Epitope-blocking enzyme-linked immunosorbent assay (ELISA) procedure to screen sera for influenza type A antibodies
BT 053.00	DNA extraction from fecal pellets using the DNeasy blood and tissue kit

CH - CHEMISTRY & CHEMICAL MGMT.

CH 001.00	Receipt, Storage, Use, and Disposal of Chemicals
CH 002.01	Calculations, significant figures and rounding
CH 003.02	Analytical chemistry chain-of-custody procedures for samples, raw data, and quality control matrices
CH 004.00	Chemical Reference Standards
CH 005.00	Laboratory Reagents
CH 006.00	Reference Standard Solutions
CH 007.00	Analytical Services Project Notebooks
CH 008.00	Pipettes, Repipettes, Pipetters, and Syringes
CH 009.00	Analytical method format
CH 010.00	Quality Control Sample Preparation and Analysis
CH 011.00	Selected Avian Tissue Preparation for Chemical Residue Analysis
CH 012.00	Preparation of Bait Samples for Analysis
CH 013.00	Sampling formulated baits for chemical analysis
CH/CO 001.01	Chemical Accountability and Tracking System
CH/CO 002.00	terminated
CH/CO 003.01	Document Control (RMA Project)
CH/CO 004.01	Internal Performance Review (RMA Project)
CH/CO 005.01	Data Review Procedure (RMA Project)
CH/CO 006.00	Control Charts (RMA Project)
CH/CO 007.01	Analytical method validation
CH/CO 008.00	terminated
CH/FL 001.01	General guidelines for safely handling chemicals at the Florida Field Station
CH/FL 002.00	Ordering, receiving, and dispensing of chemicals at the NWRC Florida Field Station
CH/FL 003.00	Chemical Accountability and Tracking System at the NWRC Florida Field Station
CH/HI 001.00	Ordering, receiving, handling, storing, and disposal of chemicals and pesticides at the Hawaii Field Station

CHEM -- WRC SOPs that are current

WRC-203	Handling, shipment, and storage of TLC plates for residue analysis
WRC-308R1	Procedures for studies of chemical photolysis on soil
WRC-317	Density, bulk density, and specific gravity
WRC-490R2	Chemical management - Millville (UT) Predator Research Facility
WRC-511	Ordering, receiving and dispensing of chemicals (Ohio)
WRC-512	Chemical accountability and tracking system (Ohio)

FP - FIELD PROCEDURES

FP 001.01	Live-Trapping Pocket Gophers
FP 002.01	Open-hole index to determine pocket gopher pre- and post-treatment activity
FP 003.00	The closed-hole method to measure pre- and post-treatment ground squirrel activity
FP 004.00	Field Determination of White Phosphorus Mortality in Waterfowl at Eagle River Flats, Alaska
FP 005.00	Ground transport of red-winged blackbirds, yellow-headed blackbirds, common grackles, and birds of similar size
FP 006.00	Postmortem examination of DRC-1339 killed birds
FP 007.00	Labeling and recovering radio instrumented and non-instrumented waterfowl at Eagle River Flats, AK
FP 008.00	Open-hole census method for moles
FP 009.00	Blood withdrawal of Canada geese-general procedures
FP 010.00	Field sample collection for survey of pathogenic E. coli
FP 011.00	Surgical implantation of miniature radio transmitters into Brown treesnakes (Boiga irregularis)
FP 012.00	Collection & processing of blood samples from small birds for virus isolation assays
FP 013.00	Medium-sized mammal capture, handling, and transport to the NWRC
FP 014.00	Two person method of drawing blood samples from birds, including syringe preparation and sample processing and storage
FP 015.00	Kill-trapping procedures for small mammals in the field
FP 016.00	Using firearms for the collection of piscivorous birds at aquaculture facilities, loafing sites and night roost
FP 017.00	Capturing rats (Rattus spp) using snap or kill traps
FP 018.00	Procedure for attachment of radio transmitters to small passerines using a two-loop figure-8 harness developed by Rappole & Tipton (1991)
FP 019.00	Use of Compact Box Trap Developed by Bray et al. (1975) for Capturing Territorial Blackbirds
FP 020.03	Collection of avian feces for wildlife disease surveillance
FP 021.00	Operation of funnel traps for small birds
FP 022.00	Collection and processing of oral swabs collected from birds for virus isolation
FP 023.00	Live-trapping prairie dogs using cage traps
FP 024.00	Visual counts of prairie dogs to obtain population estimates
FP 025.00	Adjustment of visual counts of prairie dogs for visual obstruction
FP 026.00	Sexing and aging of black-tailed prairie dogs

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FP 027.00	Assessment of the reproductive status of prairie dogs
FP 028.00	Marking individual animals with fur dye
FP 029.00	Use of a spring scale for body mass measurements
FP 030.00	Collection and processing of blood samples from prairie dogs and similar size mammals
FP 031.00	Live-trapping, transportation to research facility, handling, and processing of <i>Rattus</i> spp.
FP 032.00	Sexing and aging grey squirrels
FP 033.00	Injection of the EZ _{IP} /AVID Passive Integrated Transponder (PIT tag) for Individual Animal Identification
FP 034.00	Recovery and handling of animals found dead during DoD Environmental Security Technology Certification Program (ESTCP) aerial delivery project
FP/FL 001.00	Trapping vultures using a wa k-in funnel entrance trap
FP/HI 001.00	Anesthetizing, marking, and radio-tagging mongooses (<i>Herpestes auroguttatus</i>)
FP/HI 002.00	Anesthetizing, marking, and radio-tagging rats (<i>Rattus</i> spp)
FP/MS 001.01	Using Softcatch leg hold traps for piscivorous birds
FP/MS 002.00	Estimating Double-crested Cormorant populations at their night roost sites
FP/ND 001.00	Censusing of birds that use sunflower fields
FP/ND 002.00	Blackbird roost population estimation procedures
FP/ND 003.00	Use of mist nets for capturing blackbirds
FP/ND 004.00	Mixing and applying fluorescent particle marker to marsh roosting blackbirds
FP/ND 005.00	Mixing and applying Rodeo herbicide (glyphosate) to aquatic vegetation
FP/ND 006.00	Assessing blackbird damage to sunflower fields
FP/ND 007.00	Censusing blackbirds on quarter sections
FP/ND 008.00	Estimating bait consumption in sunflower fields
FP/ND 009.00	Examination of blackbirds for the presence of fluorescent pigment
FP/ND 010.00	Applying DRC-1339-treated bait to plots in agricultural fields
FP/ND 011.00	Roadside index of blackbirds and raptors
FP/ND 012.00	Censusing birds and mammals that use grain fields
FP/ND 013.00	Index of blackbirds and raptors using fixed-radius point counts
FP/ND 014.00	Blackbird collection procedures at roost sites and in breeding areas
FP/ND 015.00	Nine standard morphological measurements on birds
FP/ND 016.00	Counting birds at wetlands in the prairie pothole region
FP/ND 017.00	Index of birds in sunflower fields using fixed-radius points
FP/UT 001.00	Disposal of animal carcasses from the National Wildlife Research Center (NWRC) Logan Field Station
FP/WA 001.01	Live trapping mountain beavers (<i>Aplodontia rufa</i>)
FP/WA 002.01	Kill-trapping mountain beavers (<i>Aplodontia rufa</i>) for study
FP/WA 003.00	Handling mountain beavers (<i>Aplodontia rufa</i>), attaching ear tags and radio transmitters, and injecting AVID microchips
FP/WA 004.00	Live trapping free roaming dogs around captive deer pens

FIELD PROCEDURES -- WRC SOPs that are still current

WRC-23	Lubrication of M-44 ejectors prior to field use
WRC-69R6	Trapping, handling, ear-tagging, and radio-collaring ground squirrels
WRC-71R2	Radio-tracking animals equipped with miniature radio transmitters: vehicle ground-tracking system and procedures
WRC-88R1	Owl censusing technique at blackbird roosts using taped owl calls
WRC-146R2	Kill-trapping pocket gophers
WRC-210R2	Method of attaching patagial markers to medium-sized or larger birds
WRC-211R1	Procedure for hot-melt glue attachment of miniature radio transmitters to the base of tail feathers of ravens and other birds
WRC-272R2	Dosing large birds (e.g., waterfowl & gulls) with chemicals in prepared bread baits
WRC-284R1	Preparing alpha-chloralose treated grain and bread baits for field trials involving waterfowl and other birds
WRC-300R1	Criteria for evaluation of stages of anesthesia in waterfowl and pigeons dosed with alpha-chloralose
WRC-302	Procedures for conditioning coyotes to kill selected prey species
WRC-338R1	DRC-1339 treated eggs to control corvid depredations
WRC-366	Assessing rat damage in Hawaiian sugarcane
WRC-373R1	Procedure for attachment of miniature radio transmitters to the base of tail feathers of double-crested cormorants
WRC-374R1	Procedure for capture of double-crested cormorants using night-lighting technique in night roost sites
WRC-395R1	Ground transport of pocket gophers and other small mammals
WRC-460	Procedure for using pyrotechnic devices for dispersing winter roosting cormorants
WRC-500	Behavioral observations of captive coyotes or other species at the Millville Predator Research Facility
WRC-513	Conducting piscivorous bird behavioral observations at aquaculture facilities
WRC-514	Censusing piscivorous birds at aquaculture facilities
WRC-537	Handling, anesthetizing, weighing, sexing, and radio-tagging live pocket gophers

HS - HEALTH & SAFETY

HS 001.01	Chemical Spills, Exposures, and Emergencies
HS 002.00	Shipment of Dangerous Goods
HS 003.02	Respiratory Protection
HS 004.00	Personal Protective Equipment
HS 005.00	Control of Hazardous Energy

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HS 006.01	Hazardous waste collection, storage and removal
HS 007.00	Safe and effective handling of contaminated laboratory items
HS 008.00	Hazard Communication
HS 009.00	Handling Gas Cylinders
HS 010.01	Procedures for Radioisotope Monthly Radiation Surveys
HS 011.00	Radiation Safety Procedures for the RIA Laboratory
HS 012.00	Standard & special practices, safety equipment.....BSL 2 Labs
HS 013.02	Shipment of biological substances, animal specimens, and environmental test samples
HS 014.02	TERMINATED - replaced by HS 013.01
HS 015.00	Standard and special practices, safety equipment, and facility procedures for Biosafety level 3 laboratories
HS 016.00	Use of Biosafety Cabinets in Biosafety Level 3 conditions
HS 017.00	BSL-3 laboratory (Room 157) entry and exit
HS 018.00	Human and equipment entry into BSL-3 suite
HS 019.00	Human and non-autoclavable materials exit from BSL-3 suite
HS 020.00	Incident reporting procedure for the BSL-3 facility
HS 021.00	BSL-3 Laundry procedures
HS 022.00	Medical procedures for animal bites, needle sticks, and other biohazardous exposures
HS 023.00	Basic Sanitation Procedures for ARB and OARF
HS 024.00	Receiving shipments containing diagnostic specimens for the Wildlife Disease Program
HS 025.00	Protecting employee health for those who work in BSL-3 laboratories
HS 026.00	Process to obtain unescorted access to the NWRC BSL-3 facility
HS 027.00	Emergency procedures for the BSL-3 laboratory
HS 028.02	An early detection system for highly pathogenic H5N1 Avian Influenza in wild migratory birds – sample flow
HS 029.00	Pest Control
HS 030.00	Safe handling and disposal of sharps
HS 031.00	Preparing samples for shipment to the NWRC Analytical Chemistry Project for analysis
HS 032.00	Procedure for biohazardous waste disposal in Biosafety Level-2 laboratories
HS 033.00	Decontamination procedures for Transmissible Spongiform Encephalopathies (TSEs) at the National Wildlife Research Ctr. Fort Collins, CO
HS 034.00	Chain of custody procedures for government firearms
HS 035.00	Safety procedures for working with venomous snakes at NWRC
HS/MS 001.00	Hazardous Material Storage and Disposal Procedures for the Mississippi Field Station
HS/MS 002.00	Storage, labeling, retention time, and disposal of biological specimens at the Mississippi Field Station

IE - INSTRUMENTATION & EQUIPMENT

IE 001.00	pH meters and electrodes
IE 002.00	Balances
IE 003.00	Class S weights and QC check weights
IE 004.01	Dickson D200 Temperature / Humidity Data Logger
IE 005.00	Laboratory Fume Hoods
IE 006.00	Constant Temperature Circulator baths
IE 007.00	Analytical Columns
IE 008.00	Ultrasonic Cleaners
IE 009.00	Maintenance and use of centrifuges and mechanical shakers
IE 010.00	Thermometers
IE 011.00	Analytical Evaporators (N-Evaps)
IE 012.00	Construction and operation of the Modified Australian Crow Trap
IE 013.00	Security for automated data collection systems
IE 014.00	Analytical instrumentation
IE 015.00	terminated
IE 016.01	Preventive maintenance for gas chromatographs
IE 017.01	terminated
IE 018.00	Refrigerators and freezers
IE 019.00	Maintenance of liquid scintillation detector
IE 020.00	Performance verification for Geiger-Mueller Detector
IE 021.00	VWR Digital Hygrometer/Thermometer/Dew Point Unit
IE 022.00	Cole-Parmer Thermistor Thermometer Meter
IE 023.00	Use of 3M Torch
IE 024.01	terminated
IE 025.00	terminated
IE 026.01	Operation of the HOBO Temperature or HOBO Temperature and Relative Humidity Data Logger
IE 027.00	Operation of the Hotsy Hot-water high-pressure washer
IE 028.00	Operation of the ATS R4500C data logger and downloading procedures
IE 029.00	Genesys 2 Spectrophotometer
IE 030.00	Immunology Series 290 Nephelometer
IE 031.00	Zymark Rapid Trace SPE Workstation
IE 032.00	BSL-3 Autoclave procedures
IE 033.00	Use and maintenance of the Sterilmatic Autoclave
IE 034.00	Use of the Cannon-Fenske Opaque (Reverse-Flow) Viscometer
IE 035.00	Operation of the Qiagen BioRobot MDx Nucleic Acid Purifier
IE 036.00	CEM Corporation Microwave Accelerated Reaction System (MARS)
IE 037.00	003106 Operation of the Abaxis Vetscan Blood Chemistry Analyzer

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IE 038.01	Use and care of Powered Air Purifying Respirators (PAPRs) in Biosafety Level 2 and 3 conditions
IE 039.00	Operation of the Abaxis Vetscan HMT Hematology Analyzer
IE 040.00	Operation and maintenance of the Ohaus Champ II/CD-11 Indicator Scale
IE 041.00	Operation of the R.J. Harvey OX-600 Biological Oxidizer
IE 042.00	Use of metabolism cages/chambers
IE 043.00	Procedure for routine maintenance of ABI 3130 or 3130xl Genetic Analyzers
IE 044.00	Use and maintenance of cannon nets

IE/FL 001.00 Use and calibration of pocket refractometers

IE/HI 001.00 Operation of the Sears Kenmore Model 17785 Automatic dishwasher

IE/MS 001.00 Guidelines for using the Mississippi Field Station rocket-net system

IE/MS 002.00 Safe operation of outboard motorboats at the Mississippi Field Station

IE/WA 001.00 Operating and maintenance of FreeZone[®] 12 liter freeze dry system (Model # 77540)

INST. & EQUIPMENT -- WRC SOPs that are current

WRC-24	Locating radio transmitters using hand-held fixed-station and aircraft-mounted receiving equipment
WRC-33R1	Hewlett Packard Signal Generator Model 8656A
WRC-34R1	Hewlett Packard Power Meter Model 432A with Thermistor Mount
WRC-35R1	Hewlett Packard Spectrum Analyzer Model 8557A
WRC-299R1	Operation of the VWR electronic LCD digital stop watch
WRC-305R1	Temperature controlled floor model fluid bath
WRC-309R1	Freezer/Incubator
WRC-314R2	Plant Growth Chambers
WRC-326	Refrigerator and freezers temperature monitoring
WRC-334R1	Use of the net-launcher (net gun)
WRC-340R1	A teflon-ribbon body harness for back-pack transmitters on ravens and other birds of similar size
WRC-391	Avian blood collection and analysis using the Kodak Ektachem DT system
WRC-427	The LI-610 Dew Point Generation System
WRC-571	Using padded-jaw leg hold traps for capturing vultures

LP - LABORATORY PROCEDURES

LP 001.00	Formulation of toxic baits
LP 002.00	Sampling Grain Baits and Concentrates for Chemical Analysis
LP 003.00	Product Storage Stability [OPPTS 830.6317] (GDLN 63-17)
LP 004.00	Acetaminophen Tablet Preparation
LP 005.00	Chloralose Tablet Preparation
LP 006.00	Viral RNA extraction using the QIAamp Viral RNA mini spin kit
LP 007.00	Specifying color by the Munsell Coding System
LP 008.00	Preparation of teeth and/or jaw tissue samples for tetracycline biomarker and aging analysis
LP 009.00	Use of bright field and fluorescence microscopy to analyze prepared tooth and/or jaw tissue sections for tetracycline biomarker and age;
LP 010.01	Laboratory biosafety for <i>in vitro</i> research using prions of non-BSE and non-Human origin.
LP 011.01	Necropsy removal of spleen and brain from BSL-2 TSE infected mice or other rodents
LP 012.00	Preparation and analysis of bird esophagus and gizzard contents for DRC-1339 colorimetric analysis and evaluation of consumption
LP 013.00	Deer necropsy procedure for contraceptive studies
LP 014.00	Preparation of chlorophacinone in propylene glycol for stock gavage solutions
LP 015.00	Gavage of small mammals (≤ 2 kg)
LP 016.00	Preparation of DiazaCon-treated feed for Coturnix quail
LP 017.00	Preparation of birds for tissue analysis of test material residues
LP 018.00	Evaluation of Rhodamine B in whiskers using a handheld UV lamp and a fluorescent microscope
LP/HI 001.00	Recovering, drying, and weighing test food and calculating corrected consumption for rodent bioassays at the Hawaii Field Station
LP/HI 002.00	Preparation and examination of rodent (<i>Rattus</i> spp.) stomach contents under a dissecting microscope

LABORATORY PROCEDURES -- WRC SOPs that are still current

WRC-15R1	Surgical procedures, including pre-surgical preparations, laparotomy, tubal ligation, vasectomy, and post surgical care for coyotes
WRC-59R3	Small mammal ranking by weight for testing
WRC-123R1	Shipping tissue samples
WRC-143R1	Assaying animal tissues for presence and identification of radioactive markers
WRC-199R1	San Diego Instruments two-unit startle response system: Norway rat, Prairie dog, and Rock Dove Response Tests
WRC-208	Standard protocol for evaluation of repellent effectiveness with birds
WRC-246R1	Assignment of rats (<i>Rattus</i> spp.) to treatment groups
WRC-286R1	Computerized random selection of test animals and assignment of treatment groups
WRC-343R1	Dosing birds with chemicals in grain baits
WRC-438	Preparing strychnine paste concentrations

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WRC-439R1 Preparing alfalfa baits
WRC-530 Germination of test rice seed to determine viability

From: [Fagerstone, Kathleen A - APHIS](#)
To: [Keirn, Gail M - APHIS](#); [Eisemann, John D - APHIS](#); [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Clark, Larry - APHIS](#)
Subject: RE: APHIS Twitter Report - fyi
Date: Monday, April 22, 2013 9:35:56 AM
Attachments: [image001.png](#)

Wow—thank you for sending this. It does make you think about monitoring what you say very carefully.

Kathy

From: Keirn, Gail M - APHIS
Sent: Friday, April 19, 2013 2:32 PM
To: Eisemann, John D - APHIS; Fagerstone, Kathleen A - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Cc: Clark, Larry - APHIS
Subject: APHIS Twitter Report - fyi

FYI - Always good to remember that our emails are FOIA-able. Below is a link that was posted on Twitter by someone opposed to APHIS research with bison. It includes an email string among John Eisemann, Kathy Fagerstone, Matt McCollum, Pauline Nol, Jack Rhyan and others discussing the GonaCon-bison study.

I don't see anything that causes alarm. Everyone's responses seem to be professional. Just a heads-up...

GAIL KEIRN

Legislative and Public Affairs
USDA-APHIS-WS National Wildlife Research Center
4101 LaPorte Avenue, Fort Collins, CO 80521
Desk: 970-266-6007 | Fax: 970-266-6010
www.aphis.usda.gov/wildlife_damage/nwrc/



[Join the APHIS Stakeholder Registry Today](#)

Baby Finch @babyfinch12h

FOIA docs posted by BuffaloFieldCampaign re: disturbing US Wildlife Services **APHIS** experiments done w/our tax dollars <http://tinyurl.com/cd788vx>



From: [Eisemann, John D - APHIS](#)
To: [Fagerstone, Kathleen A - APHIS](#); [Keirn, Gail M - APHIS](#); [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Clark, Larry - APHIS](#)
Subject: RE: APHIS Twitter Report - fyi
Date: Monday, April 22, 2013 9:46:04 AM
Attachments: [image001.png](#)

Thank you for sending this to us.

John Eisemann

USDA APHIS Wildlife Services
National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
970-266-6158

From: Fagerstone, Kathleen A - APHIS
Sent: Monday, April 22, 2013 9:36 AM
To: Keirn, Gail M - APHIS; Eisemann, John D - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Cc: Clark, Larry - APHIS
Subject: RE: APHIS Twitter Report - fyi

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Kathy

From: Keirn, Gail M - APHIS
Sent: Friday, April 19, 2013 2:32 PM
To: Eisemann, John D - APHIS; Fagerstone, Kathleen A - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Cc: Clark, Larry - APHIS
Subject: APHIS Twitter Report - fyi

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I don't see anything that causes alarm. Everyone's responses seem to be professional. Just a heads-up...

GAIL KEIRN

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www.aphis.usda.gov/wildlife_damage/nwrc/



[Join the APHIS Stakeholder Registry Today](#)

Baby Finch @babyfinch12h

FOIA docs posted by BuffaloFieldCampaign re: disturbing US Wildlife Services **APHIS** experiments done w/our tax dollars <http://tinyurl.com/cd788vx>



From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Billing address for card
Date: Thursday, January 16, 2014 10:50:22 AM

(b) (6)

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Thursday, January 16, 2014 9:33 AM
To: Frey, Rebecca K - APHIS
Subject: Billing address for card

Hey Becky,
Is your billing address for your card (b) (6) . or is it (b) (6) ?
Thanks!
Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: BioPRYN Report for Bison & Buffalo
Date: Wednesday, February 05, 2014 1:34:50 PM

The first 46 are from when Pauline and I went up to MT a couple weeks ago. The last six are from the animals here that we collected blood from last week.

From: Rhyan, Jack C - APHIS
Sent: Wednesday, February 05, 2014 12:45 PM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: FW: BioPRYN Report for Bison & Buffalo

FYI. Date of collections?

From: BioTracking Testing Lab [<mailto:testinglab@biotracking.com>]
Sent: Wednesday, February 05, 2014 11:46 AM
To: Rhyan, Jack C - APHIS
Subject: BioPRYN Report for Bison & Buffalo

Dear BioPRYN Customer,

Here is the report on the samples we received from you. It's attached as an HTML file, and you should be able to directly open the attachment by double-clicking on it.

For up-to-date schedule and pricing information please visit our website at www.biotracking.com and click on Lab Services. Holiday closures and schedules are posted under Lab Services as well. We recommend checking the schedule prior to shipping samples to ensure you will receive results when expected. If you have any questions regarding the schedule, please do not hesitate to call and ask.

As always, we stand by our products and services, so please contact us here with any questions or comments you may have.

Thank you,

Amber Merk

Director of Laboratory Service & Sales

BioTracking LLC

1150 Alturas Dr. Ste. 105

Moscow, ID 83843

Office: 208.882.9736

Cell (b) (6)

amerk@biotracking.com or testinglab@biotracking.com

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From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Bison available for you or slaughter
Date: Friday, June 05, 2015 7:48:00 AM

Hey Becky,

So we will hold off on bringing animals back next week. I think Ryan is working on getting more hay in a timely manner. Hopefully we will be able to delay long enough to do a handoff later in June. See you on Monday. Let me know if there is anything I need to bring and what time y'all want to start.

Pauline

From: Frey, Rebecca K - APHIS
Sent: Thursday, June 04, 2015 9:17 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: Bison available for you or slaughter

Here is what I know of the bison I am getting rid of.

There is 1-2 year old, female that is Negative. She is from the GonaCon pen, out of a sentinel cow. I have to keep the other one since one of our sentinels for the 2nd control pen just died.

There are 2 likely negative yearling males, and 1 female from the GonaCon pen. I thought I had more females than male yesterday...oops. They have not been exposed to anything that has cultured positive so I expect them to still be negative. One of the males is from the 1st treatment animal that calved Red 02.

I also have 4 yearling females that are likely positive after being exposed to all of the abortions in the control pen at SNS. One from the only negative sentinel we have left, and 3 from known positive cows. Red 06 did not calve in 2013, had a calf last year, aborted this year. Red 13 has had 3 live births, cultured brucella in 2013. Red 22 has had 2 live births, waiting on number 3, and has not cultured brucella in any year.

Rebecca Frey
Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Bison available for you or slaughter
Date: Monday, June 22, 2015 8:15:30 AM

So, the results are in and there are 4 yearling reactors F, 1-2yo negative F, and 3 yearling negatives 2M, 1 F.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Friday, June 05, 2015 7:48 AM
To: Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Bison available for you or slaughter

Hey Becky,

So we will hold off on bringing animals back next week. I think Ryan is working on getting more hay in a timely manner. Hopefully we will be able to delay long enough to do a handoff later in June. See you on Monday. Let me know if there is anything I need to bring and what time y'all want to start.

Pauline

From: Frey, Rebecca K - APHIS
Sent: Thursday, June 04, 2015 9:17 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: Bison available for you or slaughter

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There is 1-2 year old, female that is Negative. She is from the GonaCon pen, out of a sentinel cow. I have to keep the other one since one of our sentinels for the 2nd control pen just died.

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Rebecca Frey
Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Rebecca K Frey](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#)
Subject: Re: bison contraception protocol revision
Date: Friday, December 10, 2010 9:47:00 AM

I looked at the distance to the cattle, and it looks like Brogan's and SlipnSlide are 1 mile from any cattle. However, if we get into the situation where we can no longer use SlipnSlide (FS trade etc....) Franklin's southernmost fenceline is only about 1/2 mile from the northernmost fenceline of Jerry Thomas's cattle. I think we can mitigate this by putting only seronegative animals at Franklin's; but this is all contingent on lease agreements for the future.....

Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell

□ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

12/01/2010 05:41 PM

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subjectbison contraception protocol revision

All,

I revised the protocol following a conversation with Jack E. et al. The questions they asked were: 1. why collect the critters a year in advance. 2. What if we don't get 45? 3. Abortion time frame (how long til we observe it and what will we do to mitigate spread of the organism to surrounding cattle ranches. 4. More detail on transponders to detect calving/proximity. 5. How far to nearest cattle? I tried to address these. Truth is we don't know exactly how we'll detect proximity to aborted fetuses but we're working on it. Please make any changes needed and check for accuracy. (1 mile to cattle??)

Thanks much,

Jack

[attachment "ImmunocontBisonProject_12-1.doc" deleted by Rebecca K Frey/MT/APHIS/USDA]

From: [Rebecca K Frey](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#)
Subject: Re: bison contraception protocol revision
Date: Monday, December 06, 2010 8:44:00 AM

Hey,

Just got to look this over. I think it is fine but I will check the mileage out tomorrow, have to go down for IBMP anyway. Also, we still have the security cameras at Brogan's that might also be useful for monitoring activity, we may need to adjust them so that they cover more of the the field instead of the perimeter though....

Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell

Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

12/01/2010 05:41 PM

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

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Thanks much,

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[attachment "ImmunocontBisonProject_12-1.doc" deleted by Rebecca K Frey/MT/APHIS/USDA]

From: [Jack C Rhyan](#)
To: [Rebecca K Frey](#)
Cc: [Matt McCollum](#); [Pauline Nol](#)
Subject: Re: bison contraception protocol revision
Date: Friday, December 10, 2010 2:06:00 PM

Perfect. Thanks, Becky.
Jack

 Rebecca K Frey---12/10/2010 09:47:33 AM---I looked at the distance to the cattle, and it looks like Brogan's and SlipnSlide are 1 mile from any cattle. However, if we

**Rebecca K
Frey/MT/APHIS/USDA**

12/10/2010 09:47 AM

ToJack C Rhyan/CO/APHIS/USDA@USDA
ccMatt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA
SubjectRe: bison contraception protocol revision



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Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

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 Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

12/01/2010 05:41 PM

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
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Jack

[attachment "ImmunocontBisonProject_12-1.doc" deleted by Rebecca K Frey/MT/APHIS/USDA]

From: [Stephens, Stephanie H - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: bison contraception protocol
Date: Tuesday, October 04, 2011 11:32:42 AM

Hi Pauline-Is the attached still the most recent version of the GonaCon bison protocol?

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: Nol, Pauline (APHIS)
Sent: Thursday, September 08, 2011 2:10 PM
To: Eisemann, John D (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: bison contraception protocol

Hi,
Here is the updated version of the Montana Gonacon protocol since the other one is very old.
Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: Eisemann, John D (APHIS)
Sent: Thursday, September 08, 2011 10:47 AM
To: Nol, Pauline (APHIS); Stephens, Stephanie H (APHIS)
Subject: FW: bison contraception protocol

Pauline, I will look at your protocol today (before 1pm). I am forwarding it to Stephanie. She is the NEPA person for VS bison work. I will look at the NEPA portion from the NWRC Protocol perspective.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: Pauline Nol [mailto:pauline.nol@aphis.usda.gov]

Sent: Tuesday, March 08, 2011 8:36 AM
To: John D Eisemann
Cc: Jack C Rhyan
Subject: bison contraception protocol

Hi John,

I think (other than a few nitty gritty things I need to fill in like references) that I've reached my limit on competence in filling out the protocol for the bison study.

Would you be able to take a look at this, especially NEPA and material appendices? Or send me in the right direction on who can help me with this?

Thanks!

Pauline

(See attached file: AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

From: [Nol, Pauline \(APHIS\)](#)
To: [Eisemann, John D \(APHIS\)](#); [Stephens, Stephanie H \(APHIS\)](#)
Subject: RE: bison contraception protocol
Date: Thursday, September 08, 2011 2:09:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft_8_29_11.docx](#)

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1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) X <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: June 2, 2011
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date: October 1, 2021

Anticipated Project Outcome: ☒ Manuscript
☒ Report
☐ Other: _____

Materials to be archived to close this activity: Raw data
Final Report

Description of Project and NWRC Activities and Participation: This study is not part of an NWRC Project.
NWRC's role in this study will be to provide GonaCon and to run ELISAs to determine anti-GnRH titers.

Comments:

Attachments: IACUC Protocol Approval

(e.g. Material
Transfer Form,
IACUC approval,
etc.)

Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011

Proposed Experimental Termination Date: October 1, 2017

Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

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6. Related Protocols

QA-1112 GonaCon Immunocontraceptive Vaccine for White-tailed Deer (*Odocoileus virginianus*): Pivotal target animal safety study

Pivotal field study of GonaCon

immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland

QA-1417 Pivotal field study of GonaCon immunocontraceptive vaccine for use in the

contraception of white-tailed deer in New Jersey Collection of ancillary data on GonaCon

QA-1445 Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (*Dama dama*) at Point Reyes National Seashore, California

QA-1523 Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado

QA-1657 Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota

Chemical sterilization of black-tailed deer

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancies in female *Brucella abortus*-positive bison
3. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

Commented [jde1]: For the Experimental Use Permit (EUP), you will want to include a map showing the test site location and the layout of the pens (including size)

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ ml on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Pregnancy and calving/abortion rates will be documented throughout the study.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Serology (ELISA) will also be conducted at NWRC to measure antibodies against GnRH.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1 st replicate			
2012	Collect bison for 1 st and 2 nd replicate	Vaccination	Preg check	Preg check

2013	Collect bison for 2 nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies

BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d.
 - e.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx	
A. Salary and Benefits				
B. Facilities (in addition to existing facility or space costs)				
C. Equipment				
D. Supplies				
E. Animal Care Costs				
F. Operating Costs (travel, misc. services, etc)				
TOTAL	\$0	\$0	\$0	

Commented [pn2]:

Commented [jde3]: Cost?

14. Human Health and Safety

HS004-00	Personal protective equipment
----------	-------------------------------

15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. - J Wildl Dis. 34:582-9.

Yoder, C. A., and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☒ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☒ NEPA and ESA Appendix
 - ☒ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: (include appropriate title) _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart

Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study.

However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

14) Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_ See attached____

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes

Commented [jde4]: You should be able to provide the names for contacts at a number of state and federal entities involved in bison management, particularly those involved in this study

Commented [jde5]: This is the person who manages the corrals where the bison will be kept

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRh/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided

to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [Stephens, Stephanie H - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: bison contraception protocol
Date: Tuesday, October 04, 2011 11:57:25 AM

Thanks, Pauline.

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: Nol, Pauline - APHIS
Sent: Tuesday, October 04, 2011 11:34 AM
To: Stephens, Stephanie H - APHIS
Subject: RE: bison contraception protocol

Hi Stephanie,
Yes this would be the latest version.
Thanks!
Pauline

From: Stephens, Stephanie H - APHIS
Sent: Tuesday, October 04, 2011 11:33 AM
To: Nol, Pauline - APHIS
Subject: RE: bison contraception protocol

Hi Pauline-Is the attached still the most recent version of the GonaCon bison protocol?

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: Nol, Pauline (APHIS)
Sent: Thursday, September 08, 2011 2:10 PM
To: Eisemann, John D (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: bison contraception protocol

Hi,
Here is the updated version of the Montana Gonacon protocol since the other one is very old.
Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521

Phone: (970) 266-6126

Mobile: (b) (6)

From: Eisemann, John D (APHIS)
Sent: Thursday, September 08, 2011 10:47 AM
To: Nol, Pauline (APHIS); Stephens, Stephanie H (APHIS)
Subject: FW: bison contraception protocol

Pauline, I will look at your protocol today (before 1pm). I am forwarding it to Stephanie. She is the NEPA person for VS bison work. I will look at the NEPA portion from the NWRC Protocol perspective.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: Pauline Nol [<mailto:pauline.nol@aphis.usda.gov>]
Sent: Tuesday, March 08, 2011 8:36 AM
To: John D Eisemann
Cc: Jack C Rhyan
Subject: bison contraception protocol

Hi John,
I think (other than a few nitty gritty things I need to fill in like references) that I've reached my limit on competence in filling out the protocol for the bison study.
Would you be able to take a look at this, especially NEPA and material appendices? Or send me in the right direction on who can help me with this?
Thanks!
Pauline

(See attached file: AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx)


Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

From: [Pauline Nol](#)
To: [Luke J Wagner/CO/APHIS/USDA](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#)
Subject: Re: Bison contraceptive proposal
Date: Friday, June 11, 2010 3:21:00 PM
Attachments: [Wagner_Proposed Projectmmandpn.docx](#)

Hey Luke,
Overall looks good. Made a few changes to take or leave.
Thanks for doing this and have a great weekend!
Pauline

(See attached file: Wagner_Proposed Projectmmandpn.docx)

Pauline Nol, DVM, MS, PhD
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

 [Luke J Wagner---06/09/2010 12:41:26 PM---Hey all, I finished a draft of the contraception study proposal. Take a look and let me know what you think.](#)

**Luke J
Wagner/CO/APHIS/USDA**

06/09/2010 12:41 PM

To: [Jack C Rhyan/CO/APHIS/USDA@USDA](#),
[Pauline Nol/CO/APHIS/USDA@USDA](#),
[Matt McCollum/CO/APHIS/USDA@USDA](#)

cc

Subject: Bison contraceptive proposal

Hey all,

I finished a draft of the contraception study proposal. Take a look and let me know what you think.

[attachment "Proposed Project.doc" deleted by Pauline Nol/CO/APHIS/USDA]

Luke Wagner
USDA APHIS VS
(970) 494-7233 - desk
(b) (6) - cell

Proposed Project:

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids~~sw~~, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, ~~a dam~~females often ~~experiences~~experiences abortion. Subsequent pregnancies may result in abortion or the birth of a weak or normal calves and ~~may~~may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore is primarily dependant on the occurrence of pregnancy and abortion or calving in infected animals.

Objectives:

1. Evaluate the effect of immunocontraception of seropositive female bison has on *B. Abortus* transmission in a bison herd
2. Evaluate the effect immunocontraception resulting in a vaccine-induced prolonged period of anestrus has on *B. abortus* colonization in female bison
3. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition site
4. Evaluate latent infection in calves born to and reared by and suckled on *B. abortus*-seropositive bison
5. Limited-evaluation of *B. abortus* transmission to bison bulls during rut.

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Research Plan:

This general research plan will be followed; details will be worked out in further consultation with collaborators and a more extensive protocol developed. Two pastures with one herd each will be used in this study. This will take place at Brogan's (?) ranch near Gardiner, MT due to their current availability and ability to house bison. The study is anticipated to start in spring of 2011 and go for at least 3 years. This study will use 40 non pregnant heifers (seropositive and seronegative) and 4 bulls.

Pasture A will contain 10 seropositive vaccinates, 10 seronegative non-vaccinates (sentinels) and 2 seronegative bulls.

Pasture B will contain 10 seropositive non-vaccinates, 10 seronegative non-vaccinates (sentinels) and 2 seronegative bulls.

Over the 3 year period, calving, abortion results, and ~~seroconversion to brucella seropositive serology~~ in the sentinel groups will be monitored. At the end of the study, necropsy and culture of ~~the seropositive vaccinates and non-vaccinates~~ all adult animals will occur.

Commented [pn1]: We will have to do this to verify status of originally negative sentinels

Expected outcomes:

1. Determine the effectiveness of GonaCon™ in reducing transmission of *B. abortus* in Bison herds.
2. Determine the effect ~~long periods of prolonged~~ anestrus has on the transmission of *B. abortus* in bison herds.
3. Determine the risk ~~and extent~~ of exposure ~~of~~ bison herds to *B. abortus* at parturition sites.
4. Determine ~~latent~~ nature of infection in calves due to suckling of seropositive cows
5. Determine ~~the risk of venereal~~ transmission ~~factor of~~ *B. abortus* to bull bison ~~due to breeding~~.

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Commented [pn2]: Is this term palatable to you all? Or does it not do the trick?

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From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: Bison GonaCon EA - Draft Final Document
Date: Thursday, January 19, 2012 11:45:00 AM
Attachments: [1.18.12 GnRH EA FINAL_PNol edits.docx](#)

It looks good to me too. I did some really dinky editing (the doc is attached) and have two comments regarding maybe mentioning having an approved ACUC protocol and the other making sure that it is clear we will try to salvage genetics from both nonvaccinates and vaccinates if possible. The text does say that but it is not entirely clear.

Matt, are you proposing keeping some of those animals to collect embryos from, or just collecting semen and ovaries when they get euthanized?

P

From: McCollum, Matthew P - APHIS
Sent: Thursday, January 19, 2012 11:13 AM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: Bison GonaCon EA - Draft Final Document
Looks good.

Just one comment about the disposition of seropositive animals and GnRH treated animals... If we are still in the game of embryo transfer, they could be valuable as donors.

Matt

From: Rhyan, Jack C - APHIS
Sent: Thursday, January 19, 2012 10:01 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: Bison GonaCon EA - Draft Final Document
This is a little long but if you all can get a chance to skim thru it today, it would be good. It goes out tomorrow.

Jack

From: Stephens, Stephanie H - APHIS
Sent: Thursday, January 19, 2012 9:20 AM
To: Rhyan, Jack C - APHIS
Subject: Bison GonaCon EA - Draft Final Document
Hi Jack-Attached is the draft final EA for the GonaCon bison study in Montana. We're doing some final reference checks and an editorial review today.
The whole document will be ready to transmit to Ryan Clarke tomorrow for publication in Montana newspapers. If you have time to review the attached before tomorrow and you have any comments or concerns, please let me know.
I've spoken with Ryan and he will handle putting announcements in local newspapers and on the IBMP website. Deb Donch will arrange to get the EA posted on the VS brucellosis website. All comments will go to an e-mail address I've set up: eacommments2012@aphis.usda.gov.
We'll announce a 30-day comment period. I've also attached the draft legal notice, which is what will actually get published in newspapers.
Let me know if you have questions about any of this process.
Thanks,
Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134



**Evaluation of GonaCon™,
an Immunocontraceptive
Vaccine, as a Means of
Decreasing Transmission
of *Brucella abortus* in
Bison in the Greater
Yellowstone Area**

**Environmental Assessment,
January 2012**

Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of *Brucella* *abortus* in Bison in the Greater Yellowstone Area

**Environmental Assessment,
January 2012**

Agency Contact:

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I. Introduction

A. Background

In Yellowstone National Park (YNP), wild and free-ranging bison (*Bison bison*) are critical parts of a fully-functioning ecosystem as well as being important to the identity of the park. The bison are a part of the esthetic, cultural, and natural environment of the YNP. -YNP bison are chronically infected with brucellosis, a contagious disease that the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA/APHIS/VS) is striving to eliminate.

Brucellosis is a serious disease of livestock and wildlife that has significant animal and public health and international trade consequences. The disease is caused by bacteria of the genus *Brucella*. -Brucellosis occurs primarily in cattle, bison, and swine; however, cervids, goats, sheep, and horses are also susceptible.- In cattle and bison, the specific disease organism of concern is *Brucella abortus* (*B. abortus*).

In its principal animal hosts, brucellosis causes loss of young through spontaneous abortion or birth of weak offspring, reduced milk production, and infertility. In cattle and bison, the disease localizes in certain lymph nodes, reproductive organs and/or the udder, causing spontaneous abortions in females and systemic effects in both male and female animals. Weight loss and lameness may also be associated with brucellosis infection.

The shedding¹ of *B. abortus* through the reproductive tract during an abortion or calving event may contribute to the transmission of infection to other animals that come in contact with the expelled bacteria now in the environment.- Studies have shown that *Brucella* can persist on fetal tissues, vegetation and soil in YNP for as long as 81 days depending on environmental conditions (Aune et al., 2011). Spread of the disease occurs when the cattle and bison, which are social animals, sniff and lick a newborn calf, the afterbirth, and even an aborted fetus. This behavior provides an avenue for the disease to spread if *B. abortus* organisms are present. Additionally, *B. abortus* is present in the milk from infected females and can be transmitted to calves through suckling. There is no effective means of treating brucellosis in livestock or wildlife.

Studies investigating the prevalence of brucellosis in YNP bison have estimated that between 40% and 60% of YNP bison have been exposed to

¹ For purposes of the proposed study, “shedding” is to expel *B. abortus* from the body through the reproductive tract.

the disease. Further testing of animals that are seropositive² demonstrates that more than 40% of the seropositive animals are culture-positive, confirming actual infection with *B. abortus* (Meyer and Meagher, 1995; Cheville et al., 1998). In the areas outside the borders of YNP where livestock such as cattle are often raised, there is a concern that infected bison may transmit the disease to livestock if infected bison abort or calve.

Multiple Federal and state agencies³ have participated in efforts to control the potential spread of brucellosis and conserve bison through the 2000 Interagency Bison Management Plan (IBMP) (MDoL and MFWP, 2000). In 1934, a federal brucellosis program was established as part of an effort to safeguard domestic livestock (See http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/ for additional information regarding USDA APHIS' brucellosis program).

Safeguarding measures, such as preventing, detecting, and eliminating animal diseases, help to maintain the U.S. cattle industry's national and international trade interests, ensure food safety, and protect public health. The efforts of the national brucellosis program have nearly eradicated brucellosis from domestic cattle and bison populations. As of July 2009, all 50 States had attained Class-Free (disease-free) status for brucellosis in domestic cattle and bison (USDA APHIS, 2010a). Currently, the last known reservoir of bovine brucellosis is in the wild bison and elk population in the Greater Yellowstone Area (GYA). Prevention of the spread of brucellosis between infected wildlife and livestock continues to be an issue of concern. The proposed study discussed in this environmental assessment (EA) is designed to investigate the feasibility of using an immunocontraceptive vaccine, GonaCon™, as a non-lethal management option to decrease the potential risk of disease transmission by brucellosis-infected bison.

In humans, brucellosis is often referred to as undulant fever because it persists for several weeks or months and may get progressively worse if untreated. Humans are most commonly infected by consumption of unpasteurized dairy products produced from milk of infected animals, or they may become infected through direct contact with infected animal tissues such as aborted fetuses or reproductive materials. In humans, brucellosis initially causes flu-like symptoms that are treated with a rigorous course of antibiotics. In some isolated cases, the disease may develop into a variety of chronic conditions, including arthritis. Potential

² Bison that test positive on blood tests for brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative.

³ U.S. Department of Interior National Park Service (NPS); U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS); U.S. Department of Agriculture Forest Service (FS); Montana Department of Livestock (MDoL); and Montana Fish, Wildlife and Parks (MFWP).

effects of the proposed study on humans will be discussed in the potential environmental impacts section.

GonaCon™ Immunocontraceptive Vaccine

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GonaCon™ is a contraceptive vaccine that stimulates an immune response in a vaccinated animal by producing antibodies that bind to a gonadotropin-releasing hormone (GnRH). GnRH is a naturally occurring hormone that signals production of sex hormones such as estrogen, progesterone, and testosterone. The anti-GnRH antibodies interfere with the ability of GnRH to signal production of sex hormones, resulting in temporary infertility. As long as adequate levels of anti-GnRH antibodies are present in the vaccinated animal, sexual activity, breeding, and reproduction are extremely unlikely.

GonaCon™ is currently approved under the United States Environmental Protection Agency's (EPA's) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for use in female white-tailed deer as one tool to aid in reducing deer overpopulation (EPA Registration Number 56228-40). The immune response that causes temporary infertility in deer is accomplished with a single-shot vaccine. The length of time that a vaccinated female deer remains infertile depends on the individual animal, but some pen studies have shown that 4 out of 5 female deer remain infertile for 5 years (Miller et al., 2008a). Field studies have demonstrated lower rates of infertility ranging from 88% and 47% the first and second year after vaccination, respectively (Gionfriddo et al., 2009) to 67% and 43% the first and second year after vaccination, respectively (Gionfriddo et al., 2011a).

GonaCon™ is not currently registered for use in bison. However, USDA conducted a small pilot study of penned bison and found that none of the 6 females vaccinated with GonaCon™ became pregnant the first year after treatment (Miller et al., 2004). In 2011, APHIS received approval from EPA to use GonaCon™ in female bison in the confined experimental use scenario discussed in this EA. Should the proposed study discussed in this EA proceed, the data obtained from it could potentially be used to add to the required data set needed for EPA to register the GonaCon™ vaccine for use in bison. However, the purpose for registering GonaCon™ in bison would not be for reducing overpopulation. The intended purpose of using GonaCon™ in female bison would be to manage reproduction in bison known to be infected with brucellosis by inducing temporary infertility, thereby decreasing the potential for transmission of brucellosis through abortion and calving events.

B. Purpose of and Need for the Proposed Action

The purpose of the proposed action is to conduct a study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by preventing pregnancy, calving, and abortion, thereby preventing transmission of *B. abortus*. The major objectives of the proposed study are:

- To evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in *B. abortus*-infected female bison;
- To evaluate the effect on shedding by *B. abortus*-infected female bison that are rendered temporarily infertile by GonaCon™; and
- To evaluate the effect the infertility produced by GonaCon™ has on the long-term survivability of *B. abortus* in infected female bison.

Use of an effective immunocontraceptive such as GonaCon™ to prevent pregnancy and eliminate the potential for abortions by infected bison would break the cycle of transmission of brucellosis. If female bison known to be infected with *B. abortus* do not become pregnant, they would not abort. Exposure of non-infected animals to the infected tissues and fluids from aborted fetuses would therefore be reduced.

The need for the proposed study is to provide information that would be used to evaluate the use of GonaCon™ as a nonlethal method of decreasing or controlling the risk of transmission of *B. abortus* in the YNP bison population. Brucellosis is spread within the animal population primarily through contact with infected birthing tissues or aborted fetuses and through the milk of infected cows. If GonaCon™ can effectively render brucellosis-infected female bison temporarily infertile, the primary routes of disease transmission would be blocked. In combination with other appropriate disease mitigation activities, the use of GonaCon™ may be an effective tool to assist in eliminating brucellosis from the YNP bison herd over time.

USDA APHIS has determined that under the provisions of the National Environmental Policy Act (NEPA) (see 42 U.S.C. 4321 et seq.) and APHIS' National Environmental Policy Act (NEPA) implementing procedures (see 7 CFR Part 372), an EA should be prepared for these proposed actions. The availability of this EA and a 30-day comment period will be announced by publishing a notice on the APHIS brucellosis program website, the IBMP website and/or local newspapers. APHIS' decision maker for the actions described in this EA will take appropriate action after reviewing the EA, its associated analyses, public comments received, and other relevant responses and recommendations.

II. Proposed Action and Alternatives

A. No Action (the Current Situation)

The no action alternative would result in not conducting the proposed study. If the proposed study is not conducted, the utility of GonaCon™ as a non-lethal reproductive control option in bison cannot be determined. Additionally, if the use of GonaCon™ in bison is not investigated, information would not be known on whether temporary infertility induced by GonaCon™ is effective in decreasing the shedding of *B. abortus* and ultimately the transmission of brucellosis. Without the proposed study, use of the immunocontraception approach as a viable disease management tool for bison would not be evaluated, and could not be considered as a potential management tool.

B. Proposed Action

The proposed action is to conduct a multi-year study to evaluate the potential for use of GonaCon™, an immunocontraceptive vaccine, as a non-lethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy, thereby preventing abortions and risk of transmission of brucellosis to uninfected animals from contact with infected tissues and fluids from aborted fetuses.

The proposed study would include the following activities that are discussed in further detail below:

- Capturing bison in the late winter/spring of 2011, 2012, 2013, and possibly 2014;
- Transporting the captured bison by stock trailer to APHIS' bison facilities in Gardiner, Montana;
- Collecting and evaluating blood samples to determine brucellosis infection status at the beginning of the study and monitoring infection status at regular intervals throughout the study;
- Housing, caring for, and tagging (for identification purposes) animals in Gardiner, Montana facilities;
- Injecting one group of seropositive female bison with GonaCon™ beginning in the spring of 2012;
- Commingling uninfected bulls with females during breeding season, documenting breeding behavior, and testing for pregnancy for five calving seasons;
- Monitoring pregnant bison with transmitters and daily observing them for abortions, labor, and births;
- Collecting and testing blood, milk, and vaginal swabs from female bison that give birth to test for brucellosis infection status;

- Monitoring exposure to aborted fetuses by other bison and evaluating fetuses collected during the study; and
- Evaluating data collected from the study to determine whether GonaCon™ decreases the shedding of *B. abortus* in bison.

Bison for the proposed study would be acquired during the winter when they naturally exit YNP. -The capture of bison would be conducted using methods currently in use for capturing bison according to the details of the IBMP operating procedures (IBMPOP, 2009). These procedures include hazing and/or using weed-free hay to move them to a capture facility. Approximately 104 adult bison would be used in the proposed study: 24 female bison that are seronegative for brucellosis; 72 female bison that test seropositive for brucellosis; and 8 male bison (bulls) that test seronegative for brucellosis. Female bison would be yearlings, two-, and three-years of age. If temporary chemical immobilization of any animal is needed, opioid narcotics and alpha-2-adrenergics would be used by study personnel qualified in the administration of such drugs. All bison used in the study would be identified with uniquely numbered ear tags and microchip identification.

The proposed study would take place on several double-fenced pastures at facilities in the Gardiner, Montana area: the Brogan Bison Facility in Corwin Springs (60 acres), the Slip 'n Slide pasture (25 acres), and the Rigler pasture (32 acres), all of which are located north of Gardiner, Montana. All sites are within the GYA and along Highway 89. The Brogan Bison Facility, Rigler pasture, and Slip 'n Slide pastures are currently leased by APHIS VS and Montana Fish, Wildlife and Parks and are used by APHIS VS for the bison quarantine feasibility study (MFWP, 2005). These facilities were specifically designed and erected to hold bison in a quarantine environment with hay and water as needed for an extended period of time.

The study design is as follows: In spring 2012, animals would be randomly selected to go into groups of 16 to 18 seropositive cows, four to six seronegative cows, and two bulls. Two replicate test pastures would be established in 2013 and possibly 2014 if not enough animals are captured in 2013. After three to four weeks of acclimation in the test pastures, *B. abortus*-infected female bison in one of the pastures would receive GonaCon™ vaccine (containing 3,000 micrograms in 3 milliliters of an adjuvant) delivered into the muscle on each side of the neck [or hip](#). The sites of injection would be tattooed, [or otherwise marked](#) and observed for any injection reaction. Bison in the remaining pasture would not be vaccinated.

Bulls would be separated from the cows outside of the breeding season from October to July. Prior to exposure to bulls, cows would have

breeding tags⁴ attached to them to document if bulls have mounted them to breed. Following first exposure of cows to bulls in 2012, five calving seasons would be observed (2013-2017). In February of each year, cows would be pregnancy-tested and fitted with vaginal transmitters to alert investigators to abortion or calving events.

During the abortion/calving seasons (from February until August of each year), daily observation for abortions, labor, and calving events would be conducted by study investigators. Within five days of abortion or calving, the cow would be immobilized and blood, milk, and vaginal swabs would be collected for testing. If possible, the calf would also be captured and eye swabs and blood would be collected for testing.

Following an abortion, the fetus would be left at the abortion site for 24 hours to monitor exposure to other bison. The fetus would then be collected, tested, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, Montana.

Blood testing of cows, bulls, and calves would be conducted three times a year: in February, calving time, and in the fall. Blood would be analyzed at the MVDL and/or the National Veterinary Service Laboratories in Ames, Iowa throughout the study to determine *B. abortus* infection status of each animal.

Handling and physical restraint of bison for tagging or blood collection would take place in alleyways leading to standard bison manual squeeze chutes. Injection of the study animals with GonaCon™ would be done by study personnel experienced in administering intramuscular vaccines. Blood samples from study animals would be collected using established techniques for collection of blood from bison and would be performed by study personnel experienced with these techniques. An attending veterinarian would be available to address concerns about animal care and use for the study.

When the study is completed, all seropositive animals would be humanely euthanized following American Veterinary Medical Association-approved guidelines, and specimens would be collected from each animal for laboratory analysis. In addition, eggs and semen would be collected from these animals and frozen for genetic conservation. Per the conditions of the approval from EPA to use GonaCon™ in bison in this confined experimental use study, animals treated with GonaCon™ cannot be consumed by humans. These animals would be disposed of by incineration or landfill burial. Seropositive animals from the study that have not received GonaCon™ injections would be distributed to Montana food

Commented [PN1]: Maybe add here: "including vaccinated animals."

⁴ Breeding tags are devices that are temporarily adhered to the base of the cow's tail that indicate by a color change that the cow has been mounted.

banks as is routinely done with other YNP seropositive bison. Further discussion on the safety of consuming bison infected with *B. abortus* is discussed in the human health and safety section of this document. All animals that test negative for brucellosis for the duration of the study and satisfy existing bison quarantine release requirements outlined in the APHIS Uniform Methods and Rules (USDA APHIS, 2003) would be used for bison conservation purposes.

C. Other Alternatives Considered but Dismissed from Further Consideration

Because the most common route of transmission of *B. abortus* is contact with infected birthing fluids, aborted fetuses, and placental tissues, different methods of impacting the fertility of bison through the use of immunocontraceptive vaccines were considered as alternatives to the proposed action. If pregnancy could be prevented in *B. abortus*-infected female bison, transmission of *B. abortus* by this route could be eliminated or decreased.

APHIS considered the use of [pPorcine zona pellucida \(PZP\)](#), another type of immunocontraceptive vaccine that has been used in animal species such as dogs, coyotes, burros, wild horses, and deer (USDA APHIS, 2010b). PZP has also been demonstrated to effectively induce temporary infertility in captive bison (Frank et al., 2005). However, research has shown that the use of PZP can increase the period of time in which the treated animals exhibit breeding season behavior.

The PZP vaccine results in temporary infertility while still allowing female animals to have multiple estrous cycles in which they engage in prebreeding behavior and breed. This behavior can cause animals to use energy at times of the year, such as late fall and early winter, when they would otherwise be conserving energy. Miller et al. (2004) concluded that "...Prolonging the breeding season of bison in the GYA may be deleterious to the winter survival of dominant bulls and PZP vaccinated cows because of increased sexual activity during fall and early winter." Therefore, this alternative was dismissed from further consideration because investigating the use of a PZP vaccine would not be useful in brucellosis management strategies in bison since it is associated with increased mating and reproductive activity (Killian et al., 2007).

APHIS also considered the alternative of physical sterilization as a means of decreasing the transmission of *B. abortus* within bison populations and between bison and cattle in the GYA. Physical sterilization such as

spaying⁵ or castration⁶ has been recognized as a disease management strategy that could be used to reduce the potential transmission of brucellosis in infected wildlife populations. However, this type of sterilization is permanent. APHIS would not subject the bison in the study to physical sterilization. For this reason, this alternative was dismissed from further consideration.

III. Potential Environmental Impacts

The NEPA implementing regulations provide criteria that Federal agencies should evaluate, if applicable, in environmental documents for proposed actions. This section of the EA addresses the applicable criteria related to potential impacts from the no action alternative and from the proposed action. NEPA criteria that are applicable for consideration in this section of the document include animal impacts, human health and safety, and the physical environment.

A. No Action

Without the proposed action, efforts to gather scientific information to better understand the potential application of immunocontraceptive vaccines such as GonaCon™ as a nonlethal strategy for reducing the transmission of *B. abortus* and decreasing the prevalence of brucellosis in the wild bison population in YNP would be lost. Without the proposed action to assist in developing nonlethal strategies to effectively control and eliminate brucellosis, the disease may continue to spread within the wild, free-ranging bison population in the GYA.

B. Proposed Action

a. Bison

The proposed study would not increase the risk of brucellosis being transmitted within the bison population. Therefore, this section focuses on the potential effects of the administration of GonaCon™ in bison.

In this proposed study, the desired effect of administering GonaCon™ is the temporary suspension of reproductive activity in the vaccinated female bison. Miller et al. (2004) report that “The gonadotropin-releasing hormone (GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns

⁵Surgical removal of the ovaries from female bison.

⁶ Surgical removal of the testes of male bison.

as the GnRH antibody titer drops below a protective level.” If the effect of this immunocontraceptive vaccine successfully places the vaccinated bison cows in a temporary nonreproductive state, the transmission of brucellosis by the female bison via shedding of *B. abortus* during calving or abortion may be eliminated.

A small study conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho in 2002-2003 demonstrated “that a single injection of GnRH vaccine is effective in preventing conception in female bison for at least 1 yr” (Miller et al., 2004). In that study, three of the six GnRH-treated bison cows and five of the untreated bison cows were in the last month of pregnancy at the time of vaccination. They delivered normal calves in the first year, suggesting that the GnRH vaccine did not interfere with the pregnancy and could be administered safely during the last third of the pregnancy. Additionally, none of the six treated bison became pregnant during the first breeding season (Miller et al., 2004).

Undesired health effects have been minimal in the species of wildlife in which GonaCon™ has been used. Injection site reactions caused by the “water-in-oil (W/O) emulsion needed in the GonaCon™ formulation for development of a long-term immune response” have been observed (Miller et al., 2008b). These reactions were most commonly manifested as inflammation or swelling at the injection site, or the presence of granulomas (thickened tissue filled with fluid). This observation is not uncommon in other livestock vaccines (USDA APHIS, 2010b).

As part of the GonaCon™ EPA registration process for use in deer, the health effects to the vaccinated deer were evaluated. Vaccinated animals showed no external evidence of inflammation at known injection sites; however, when muscle tissue at the injections site was sectioned, the injection sites appeared to be comprised of whiteish scar tissue, some containing vesicles of sterile fluid. All blood chemistry analyses were similar between treated and untreated deer. (Killian et al., 2006). Other types of injected products that alter animal hormones are currently used in livestock in the United States (USDA APHIS, 2010b).

Ensuring humane handling and treatment of all bison during the proposed study activities would be a priority. Application of animal identification tags, administration of GonaCon™ vaccine, and evaluation of pregnancy status, calving, or abortion activities would be conducted at appropriate times during the proposed study. These activities would be overseen by the study’s attending veterinarian and would not be expected to cause more than momentary or slight pain or discomfort. All temporary restraining and handling or temporary immobilization and handling activities would be conducted as quickly and efficiently as possible and in a manner that would prevent undue stress, trauma, injury, or any

unnecessary discomfort to the animal. If temporary immobilization is necessary, bison cows would be immobilized in locations within the facilities that are safe for the animals and the proposed study personnel. Veterinary oversight for animal care and handling, restraint, and sample collection would be provided during the proposed study activities. Wildlife biologists trained and experienced in the handling of bison would also be participating in the proposed study activities.

Commented [PN2]: Do we mention here that all the work is performed under a protocol approved by an institutional animal care and use committee?

If necessary, study personnel would use the Federal Drug Administration (FDA)-approved anaesthetic and pain-killing (analgesic) drug combinations to immobilize the animals in order to prevent any potential negative impacts to the bison during the collection of study samples. The immobilization drugs would be used according to standard animal administration techniques, and it is expected that the bison would be immobilized for no more than 20 minutes. Vital signs of the immobilized bison would be monitored by qualified study staff throughout the sampling procedures and the initial recovery phase. To further ensure humane handling of the bison, every precaution would be taken by study staff to prevent immobilization- or handling-related trauma, injury, or death to the bison. The standard chemical immobilization protocol that would be used in this proposed study is widely used in bison and other wild ungulates without long-term effects (Kreeger et al., 2002).

In the GonaCon™ EPA registration process for use in deer, concerns were initially raised by some States that GonaCon™ would eliminate the need to use hunting as a tool to control deer overpopulation. Contraception alone would not reduce overabundant deer populations to healthy levels (USDA APHIS, 2010b). In deer, GonaCon™ is meant to be used in combination with other wildlife management tools to control populations. Assuming the use of GonaCon™ is eventually registered by EPA for bison, it is equally implausible to conclude that use of the contraceptive vaccine in bison would result in any significant population decreases in bison in the absence of other management tools (USDA APHIS, 2010b).

b. Non-Target Species

The proposed study would not increase the risk of brucellosis being transmitted to non-target species. Therefore, this section focuses on the risk of non-target species being exposed to GonaCon™.

In the proposed study, it is unlikely that non-target species would be exposed to GonaCon™. The proposed study protocol includes both risk mitigation measures that prevent direct exposure of non-target species to GonaCon™ and measures that limit the potential for secondary exposure of non-target species to GonaCon™.

To prevent direct exposure to non-target species, GonaCon™ would be administered directly to the study bison by hand-injection with a syringe. By using this direct-injection method, there would be no potential for accidental injection of non-target species with GonaCon™.

To prevent the risk of secondary exposure, the study plan includes measures to restrict access to treated animals by predators or other non-target species. To prevent access by larger wild animals, the bison in the proposed study would be maintained in double-fenced pastures, not on open range, thereby physically limiting potential contact between treated bison and wild animals such as elk, bears, and coyotes.

Abortions or calving events by GonaCon™-treated bison should be very minimal since the expected effect of treatment with GonaCon™ is to prevent pregnancy. The proposed study protocol includes actions to detect abortion or calving events, and the fencing would also physically limit some wild animals from accessing infected bison tissues from the GonaCon™-treated bison. The study protocol also includes standard operating procedures for proper removal and disposal of *B. abortus*-infected animal tissues from GonaCon™-treated bison from the study area to further limit potential exposure.

As discussed above, some larger animal species can be physically prevented from accessing the study area. However, some species such as birds of prey, smaller rodents, or insects cannot be prevented from accessing the study area. In the event that a non-target species were to consume GonaCon™-treated infected bison carcasses or GonaCon™-treated *B. abortus*-infected animal tissues, there would be no anticipated adverse effects from the GonaCon™ vaccine. Because GonaCon™ is made of proteins, it is broken down into smaller amino acids through digestion when it is consumed and has no contraceptive effect on non-target species (Fagerstone et al., 2008; Fagestone et al., 2010).

As part of the registration process for the use of GonaCon™ in deer, EPA concluded that there is no known danger associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007). Similar injectable hormone-altering products are used routinely in livestock applications (USDA APHIS, 2010b).

2. Human Health and Safety

a. General Public

The proposed study discussed in this EA would be conducted on double-fenced, private facilities where access by the general public to bison and potentially infected animal tissues such as aborted fetuses or reproductive materials would be prohibited. The protocol for the study contains standard operating procedures for handling and safely disposing of any

potentially brucellosis-infected materials generated from the animals in the study. The general public would have no risk of being exposed to either GonaCon™-treated or untreated animals from the study or any potentially infected materials generated from the study.

There is no danger of transmission of the infection to humans from consuming cooked meat from *B. abortus*-infected bison. The *B. abortus* bacteria that causes brucellosis is typically not found in muscle tissue, and normal cooking temperatures kill any existing bacteria (USDA APHIS, 2011). Additionally, EPA and FDA concluded that there are no known human food safety concerns associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007 and FDA, 2005).

b. Worker Safety

Personnel who would be involved in the proposed study are qualified and have the expertise and experience needed to carry out the study activities. These activities include wildlife chemical immobilization, proficiency in administration of animal vaccines, veterinary care, animal restraint, tagging and marking animals, sample collection, and field evaluation of reproductive behaviors and activities.

Standard operating procedures would be in place to protect personnel involved in carrying out the proposed study activities. The standard operating procedures would include measures for safe and humane handling of bison to prevent injury to study personnel and to bison; safe handling and administration of GonaCon™; safe and humane collection of study samples for analysis; and safe handling procedures for study samples, including the safe handling and proper disposition of potentially infected animal tissues. In addition to the standard operating procedures and safety measures, at least one cell phone would be available at all times to facilitate contact in emergencies, and first aid kits would be available at all times in the event of injury to study personnel.

The GonaCon™ immunocontraceptive vaccine would be provided for the study in pre-mixed syringes and stored in locked containers except when actively being used to inject study animals. Personnel handling the vaccine would take appropriate precautions to prevent accidental self-injection. Pregnant women would not be involved in the handling or injecting of GonaCon™ at any time during the proposed study to avoid any potential risks associated with accidental exposure to the immunocontraceptive vaccine. Immobilization drugs and associated reversal drugs would be available for use if needed in the study. These drugs would be properly stored in locked containers to prevent improper access.

3. Physical Environment

As previously mentioned, proposed study activities would occur in several pastures at the Brogan Bison Facility, just north of Corwin Springs (60 acres), and the Slip 'n Slide pasture (25 acres) and/or Rigler pasture (32 acres), located north of Gardiner, Montana.

The Brogan Bison Facility is used by APHIS VS for bison research. Forage at the pastures includes a mix of cultivated and native grasses. The upper pasture is on a steep slope along the west side of the property with several grass benchlands⁷ and steep, rocky drainages. The vegetation is composed of thinly forested slopes, interspersed with native bunchgrass rangelands (MFWP, 2005). Bassett Creek runs through the property and flows into the Yellowstone River.

The Slip 'n Slide and Rigler pastures are located in close proximity to each other, just south of Yankee Jim Canyon. The pastures are double-fenced. The landscape is gently sloping and consists mostly of native grassland, except for the mixed alfalfa- and grass-cultivated hay meadows. Slip 'n Slide Creek runs through the Slip 'n Slide property and flows into the Yellowstone River. There are no brooks or creeks running through the Rigler pastures. The pastures are primarily surrounded by Gallatin National Forest and State of Montana land. Montana Fish, Wildlife and Parks historically leases the pastures on the ranch for bison to graze on (MFWP, 2011).

The potential environmental impacts of the proposed study on the physical environment would primarily be due to bison grazing in confined areas. The main issues of concern regarding confined grazing are effects on soil, vegetation, and water quality. These aspects are discussed below.

a. Soil and Vegetation

Livestock grazing in confined pastures can negatively affect soil quality by compacting the soil or causing soil erosion due to loss of vegetation cover. With a loss of vegetation, invasive species also threaten pastures. Most studies and discussions on the impacts of grazing focus on cattle because 70% of the western United States is grazed by livestock, which is primarily composed of cattle (Fleischner, 1994). Cattle are similar to bison in that they are large generalists and ungulate herbivores that can disturb terrestrial communities; however, differences in the two animals, such as forage selection and social organization (Hartnett et al., 1997; Steuter and Hidingier, 1999), may influence their impacts on soil and vegetation.

⁷ Steps or shelves in the mountainside that are the remains of former riverbanks or lakeshores.

Bison have a stronger preference for perennial grasses than cattle. -Cattle consume a higher percentage of forbs⁸ in their diet than bison, and they use wooded areas and riparian zones more intensively than bison (Steuter and Hidingen, 1999). Due to the lower diversity of plants consumed by bison and the bison's preference for herbaceous vegetation, there may be a reduction in the abundance of dominant grasses, an increase in the survival of subordinate species, and an increase in species diversity, when compared to land grazed by cattle (Hartnett et al., 1997). -Additionally, physical disturbances that bison exhibit during non-grazing activities, such as wallowing⁹ may assist in ecodiversity (Hartnett et al., 1997).

The proposed action would not alter historic land use (for information regarding historic or cultural sites, see section below in the section on other environmental review requirements) at the pastures; therefore, overall effects to soil and vegetation would not be increased. Approximately 100 bison would be placed on 120 irrigated acres of land, averaging about one acre of land per bison. This density is expected to have only minimal impacts on the land. In addition, landowners at each ranch or facility implement management practices to minimize effects to soil and vegetation. Pasture rotation is practiced at or between facilities as necessary, so that each pasture is periodically rested and the land is not overused. Lastly, the bison at all facilities would be supplemented with hay, further limiting pasture grazing.

b. Water

GonaCon™ is a protein that is broken down within the treated bison; its metabolites would not be anticipated to be any greater than what would naturally occur. Therefore, this section focuses on other potential environmental impacts of bison grazing near water.

Potential environmental impacts from bison grazing in pastures could include a degradation of nearby water quality by manure, urine, and sediment being deposited into local waters. While bison that have access to a water body may directly deposit manure and urine into the water, wastes excreted onto land may also be transported to water bodies via leaching and surface runoff.

Grazing management practices can lessen the environmental impacts of streamside pastures. While many studies describe the impact of cattle grazing on water bodies, few studies have concentrated on the effects of native ungulates on stream health. Russell et al. (2009) states that the

⁸ Herbaceous flowering plants other than grass.

⁹ When bison roll in shallow depressions in the soil, covering themselves with dirt or mud.

proximity of cattle to the stream, the amount of time they spend by or in the stream, and the intensity and length of cattle grazing can all influence the water quality of nearby streams. -One can assume the same behaviors in bison would also impact water quality.

Bison spend less time in streams or riparian habitats than cattle (Fleischner, 1994). -Fleischner describes a study conducted in Utah regarding the feeding ecology of cattle and bison. The study noted that “cattle distribution was limited to gentle slopes near water, regardless of forage, while bison roamed widely, seemingly unaffected by slope or proximity to water.” As previously mentioned, cattle forage on a higher percentage of forbs and woody vegetation and maintain a larger breadth of diet niche than bison. Fritz et al. (1999) takes this one step further and states that a higher percentage of forbs and woody vegetation occurs in the riparian zone, so cattle are more likely to impact stream riparian zones than bison.

Fritz et al. (1999) studied the distribution and diversity of macroinvertebrates (e.g., insects, worms, snails and crayfish) in relation to bison crossings in prairie streams. The study suggests that impacts of bison on communities at the bottom of the streams was spatially limited, and that the bison may have less impact on stream communities than other studies of the impact of cattle. While comparison to cattle provides a noteworthy point of reference, it is important to point out that it is difficult to compare environmental responses with cattle versus bison due to confounding effects of site, weather, and management.

The pastures that would be utilized in the proposed study have historically been used for bison research or as livestock pastures, so deposits of manure, urine, and sediment due to the proposed study are not expected to increase the historic amount of contaminants entering the Yellowstone River. While the Brogan Bison Facility does have a creek running through it, bison do not have access to the creek. Only bison at the Slip ‘n Slide ranch would have direct, but limited, access to a creek. The access site to this creek was historically used for livestock and is at a point on the creek where the bank is shallow and covered with rocks. A shallow crossing means that bison would not have to climb up and down the bank, which would eventually cause the banks to erode. -In addition, water would be provided to the bison, limiting the time that bison would visit the creek. Lastly, because only a portion of the total number of -bison tested would be present on this pasture and those bison would spend limited time in streamside environments, the impact to water bodies is expected to be minimal.

IV. Other Environmental Review Requirements

A. Endangered or Threatened Species

Section 7 of the Endangered Species Act (ESA) and its implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of endangered or threatened species or result in the destruction or adverse modification of critical habitat. Proposed study activities would occur in pastures in southern Park County in Montana.

There are two federally listed mammals in Park County: the Canada lynx (*Lynx canadensis*) and the grizzly bear (*Ursos arctos horribilis*). Critical habitat has been designated for the Canada lynx in Park County.

Canada lynx: Areas designated as critical habitat for the Canada lynx include boreal forest landscapes that provide one or more of the following primary constituent elements for the lynx: snowshoe hares for prey; abundant, large, woody debris piles that are used as dens; and winter snow conditions that are generally deep and fluffy for extended periods of time (USDOI FWS, 2009).

Grizzly bear: In Montana, grizzly bears primarily use meadows, seeps, riparian zones, mixed shrub fields, closed timber, open timber, sidehill parks, snow chutes, and alpine slabrock habitats. Habitat use is highly variable between areas, seasons, local populations, and individuals. Grizzly recovery zones (areas identified where grizzly bear distribution is primarily within), including the Yellowstone area in northwest Wyoming, eastern Idaho, and southwest Montana (9,200 square miles), are estimated at more than 580 bears (FWS, 2011).

At all three locations, the pastures are double-fenced with an 8-foot woven wire fence and an electric high tensile fence to contain the study bison. These fences would also prevent Canada lynx and grizzly bears from entering the pastures. If Canada lynx or grizzly bears were to enter the pastures and consume GonaCon™-treated bison, there would be no effect on these species. The vaccine is made of proteins, and when consumed, is broken down into amino acids in the gastrointestinal tract, thereby having no contraceptive effect (Fagerstone et al., 2008; Fagerstone et al., 2010).

Federally-listed species and other non-target wildlife would not be directly exposed to GonaCon™ because the vaccine would be injected directly into the test bison and not administered orally in bait form. No wildlife habitat would be altered or disrupted by proposed study activities. No

helicopters would be used as part of this proposed study; therefore, no disturbance to wildlife in the surrounding area is expected. Although the study pastures occur within the designated critical habitat of the Canada lynx, the proposed study would have no effect on the primary constituent elements of that habitat and would not adversely modify it. Therefore, APHIS has determined that the proposed action would have no effect on the grizzly bear or Canada lynx.

B. Bald and Golden Eagle Protection Act

The Bald and Golden Eagle Protection Act (16 U.S.C. 668-668c) prohibits anyone, without a permit issued by the Secretary of the Interior, from "taking" bald eagles, including their parts, nests, or eggs. The Act provides criminal penalties for persons who "take, possess, sell, purchase, barter, offer to sell, purchase or barter, transport, export or import, at any time or any manner, any bald eagle ... [or any golden eagle], alive or dead, or any part, nest, or egg thereof." The Act defines "take" as "pursue, shoot, shoot at, poison, wound, kill, capture, trap, collect, molest or disturb."

There are no known bald eagle nests around the facilities; nesting areas are further down river (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.). However, golden eagle nests could be in the vicinity of the facilities, although specific nests are not known. Therefore, the proposed study is not expected to have any impact on nesting bald or golden eagles. In addition, activities occurring during the proposed study would not differ significantly from activities normally occurring at these pastures. "Eagles are unlikely to be disturbed by routine use of roads, homes, and other facilities where such use pre-dates the eagles' successful nesting activity in a given area. Therefore, in most cases ongoing existing uses may proceed with the same intensity with little risk of disturbing bald eagles" (FWS, 2007). If study personnel discover the presence of any bald or golden eagle nests in the area, this information would be reported to the Wildlife Program Manager at Gallatin National Forest.

Golden eagles have been observed flying over the Brogan Bison Facility (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.) and bald eagles could be flying in the area as well. The activities that would occur during the proposed study would not differ significantly from activities that normally occur in these pastures. Therefore, no disturbance of eagles would occur as a result of the proposed study; eagles in the area would be accustomed to these activities.

Although program personnel would remove daily any aborted calves or treated or non-treated bison that could die during the study, bald and golden eagles in the area could potentially consume these items. However, "[i]mmunocontraception vaccines provide few risks for

consumptive use of dosed wildlife; the antibodies that prevent reproduction are only one of millions of other antibodies present in animals, all of which are harmless to the organism that digests them, like any other proteinaceous food consisting of amino acids” (Fagerstone et al., 2010). Therefore, no eagles would be harmed if consumption of these items occurred.

C. Historic and Cultural Resources

In accordance with Section 106 of the National Historic Preservation Act of 1966 and its implementing regulations¹⁰, APHIS prepared a summary of the proposed project and submitted it to the Montana State Historic Preservation Office (SHPO) for consideration of potential impacts to historic resources. On November 28, 2011, APHIS received a letter of concurrence from the Montana SHPO agreeing that there were no findings of potential impacts to historic resources for the proposed study.

D. Tribal Consultation and Coordination

In accordance with Executive Order 13175: Consultation and Coordination with Indian Tribal Governments¹¹, APHIS has prepared a summary of the proposed project and provided it to 26 tribes who may have interests in YNP. In addition to the 26 identified tribes, APHIS also provided a summary of the project to all tribes located near YNP and in States adjacent to Montana who might potentially have interest in the project.

On December 19, 2011, APHIS held a conference by telephone with tribes to provide an opportunity to discuss the proposed project in more detail and discuss potential concerns that the tribes may have. Tribes that participated in the call showed an interest in the details of the project, and several requested additional information on the history of the GonaCon™ immunocontraceptive vaccine. APHIS agreed to provide background information to tribes. No tribes voiced any major concerns about the project.

APHIS will continue to conduct outreach to interested tribes and keep them updated on the activities associated with the project.

¹⁰ National Historic Preservation Act of 1966 (16 U.S.C. 470f) and implementing regulations (36 CFR §800).

¹¹ Executive Order 13175: Consultation and Coordination with Indian Tribal Governments (65 FR 67249, November 9, 2000).

V. Cumulative Impacts

This EA examines the activities associated with a proposed study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by effecting temporary infertility in bison cows and thereby preventing transmission of *B. abortus*. Activities associated with the proposed study are not expected to result in adverse cumulative effects.

In order to conduct the proposed study, approximately 96 female and 8 male bison that naturally exit YNP over the period of as many as three years would be housed at pasture locations in the Gardiner, Montana area. Some of the female animals in the study would be injected with GonaCon™, which would reduce the likelihood of pregnancy and delivery of offspring in the treated animals. Untreated females may give birth to offspring, which would increase the total number of animals associated with the study.

In August 2011, the National Park Service conducted an annual bison population estimate (NPS, 2011). According to the 2011 survey, the total bison population in YNP was estimated to be approximately 3,700 bison. This total was approximately 200 lower than the survey from the previous summer, but the decrease was “within the natural range of expectation for wild bison.”

Assuming the proposed study would result in approximately 104 bison being removed from the larger bison population of YNP, the effect of removing this number of bison over multiple years is well within the natural range of expectation for bison. This decrease in the numbers of bison in YNP is not anticipated to cause any cumulative negative effects to the overall bison population.

One of the goals of the IBMP is to manage temporal and spatial separation of bison and cattle to mitigate potential transmission of brucellosis. Currently, this is accomplished through hazing, capture, test and slaughter of seropositive animals, and vaccination of seronegative animals and a limited hunt in Montana. The proposed study may provide important information that would allow for re-evaluation and re-consideration of some of the current IBMP activities. This may result in impacts to future decision-making regarding protocols for bison habitat management, bison vaccination strategies, and bison hunt activities. IBMP activities that could be impacted include strategies to maintain appropriate bison population and distribution, should bison habitat be expanded.

VI. Agencies or Persons Contacted

U.S. Forest Service, Gallatin National Forest

Montana Fish, Wildlife and Parks

Montana State Historic Preservation Office, Montana Historical Society

USDA, Animal and Plant Health Inspection Service, Veterinary Services

USDA, Animal and Plant Health Inspection Service, Policy and Program Development, Environmental and Risk Analysis Services

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From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: Bison GonaCon EA - Draft Final Document
Date: Thursday, January 19, 2012 11:12:51 AM
Attachments: [1.18.12 GnRH EA FINALmpm.docx](#)

Looks good.

Just one comment about the disposition of seropositive animals and GnRH treated animals... If we are still in the game of embryo transfer, they could be valuable as donors.

Matt

From: Rhyan, Jack C - APHIS
Sent: Thursday, January 19, 2012 10:01 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: Bison GonaCon EA - Draft Final Document
This is a little long but if you all can get a chance to skim thru it today, it would be good. It goes out tomorrow.

Jack

From: Stephens, Stephanie H - APHIS
Sent: Thursday, January 19, 2012 9:20 AM
To: Rhyan, Jack C - APHIS
Subject: Bison GonaCon EA - Draft Final Document
Hi Jack-Attached is the draft final EA for the GonaCon bison study in Montana. We're doing some final reference checks and an editorial review today.
The whole document will be ready to transmit to Ryan Clarke tomorrow for publication in Montana newspapers. If you have time to review the attached before tomorrow and you have any comments or concerns, please let me know.
I've spoken with Ryan and he will handle putting announcements in local newspapers and on the IBMP website. Deb Donch will arrange to get the EA posted on the VS brucellosis website. All comments will go to an e-mail address I've set up: eacomment2012@aphis.usda.gov.
We'll announce a 30-day comment period. I've also attached the draft legal notice, which is what will actually get published in newspapers.
Let me know if you have questions about any of this process.
Thanks,
Stephanie

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**Evaluation of GonaCon™,
an Immunocontraceptive
Vaccine, as a Means of
Decreasing Transmission
of *Brucella abortus* in
Bison in the Greater
Yellowstone Area**

**Environmental Assessment,
January 2012**

Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of *Brucella* *abortus* in Bison in the Greater Yellowstone Area

Environmental Assessment, January 2012

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I. Introduction

A. Background

In Yellowstone National Park (YNP), wild and free-ranging bison (*Bison bison*) are critical parts of a fully-functioning ecosystem as well as being important to the identity of the park. The bison are a part of the esthetic, cultural, and natural environment of the YNP. YNP bison are chronically infected with brucellosis, a contagious disease that the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA/APHIS/VS) is striving to eliminate.

Brucellosis is a serious disease of livestock and wildlife that has significant animal and public health and international trade consequences. The disease is caused by bacteria of the genus *Brucella*. Brucellosis occurs primarily in cattle, bison, and swine; however, cervids, goats, sheep, and horses are also susceptible. In cattle and bison, the specific disease organism of concern is *Brucella abortus* (*B. abortus*).

In its principal animal hosts, brucellosis causes loss of young through spontaneous abortion or birth of weak offspring, reduced milk production, and infertility. In cattle and bison, the disease localizes in certain lymph nodes, reproductive organs and/or the udder, causing spontaneous abortions in females and systemic effects in both male and female animals. Weight loss and lameness may also be associated with brucellosis infection.

The shedding¹ of *B. abortus* through the reproductive tract during an abortion or calving event may contribute to the transmission of infection to other animals that come in contact with the expelled bacteria now in the environment. Studies have shown that *Brucella* can persist on fetal tissues, vegetation and soil in YNP for as long as 81 days depending on environmental conditions (Aune et al., 2011). Spread of the disease occurs when the cattle and bison, which are social animals, sniff and lick a newborn calf, the afterbirth, and even an aborted fetus. This behavior provides an avenue for the disease to spread if *B. abortus* organisms are present. Additionally, *B. abortus* is present in the milk from infected females and can be transmitted to calves through suckling. There is no effective means of treating brucellosis in livestock or wildlife.

Studies investigating the prevalence of brucellosis in YNP bison have estimated that between 40% and 60% of YNP bison have been exposed to

¹ For purposes of the proposed study, “shedding” is to expel *B. abortus* from the body through the reproductive tract.

the disease. Further testing of animals that are seropositive² demonstrates that more than 40% of the seropositive animals are culture-positive, confirming actual infection with *B. abortus* (Meyer and Meagher, 1995; Cheville et al., 1998). In the areas outside the borders of YNP where livestock such as cattle are often raised, there is a concern that infected bison may transmit the disease to livestock if infected bison abort or calve.

Multiple Federal and state agencies³ have participated in efforts to control the potential spread of brucellosis and conserve bison through the 2000 Interagency Bison Management Plan (IBMP) (MDoL and MFWP, 2000). In 1934, a federal brucellosis program was established as part of an effort to safeguard domestic livestock (See http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/ for additional information regarding USDA APHIS' brucellosis program).

Safeguarding measures, such as preventing, detecting, and eliminating animal diseases, help to maintain the U.S. cattle industry's national and international trade interests, ensure food safety, and protect public health. The efforts of the national brucellosis program have nearly eradicated brucellosis from domestic cattle and bison populations. As of July 2009, all 50 States had attained Class-Free (disease-free) status for brucellosis in domestic cattle and bison (USDA APHIS, 2010a). Currently, the last known reservoir of bovine brucellosis is in the wild bison and elk population in the Greater Yellowstone Area (GYA). Prevention of the spread of brucellosis between infected wildlife and livestock continues to be an issue of concern. The proposed study discussed in this environmental assessment (EA) is designed to investigate the feasibility of using an immunocontraceptive vaccine, GonaCon™, as a non-lethal management option to decrease the potential risk of disease transmission by brucellosis-infected bison.

In humans, Brucellosis is often referred to as undulant fever because it persists for several weeks or months and may get progressively worse if untreated. Humans are most commonly infected by consumption of unpasteurized dairy products produced from milk of infected animals, or they may become infected through direct contact with infected animal tissues such as aborted fetuses or reproductive materials. In humans, brucellosis initially causes flu-like symptoms that are treated with a rigorous course of antibiotics. In some isolated cases, the disease may develop into a variety of chronic conditions, including arthritis. Potential

² Bison that test positive on blood tests for brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative.

³ U.S. Department of Interior National Park Service (NPS); U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS); U.S. Department of Agriculture Forest Service (FS); Montana Department of Livestock (MDoL); and Montana Fish, Wildlife and Parks (MFWP).

effects of the proposed study on humans will be discussed in the potential environmental impacts section.

GonaCon™ Immun contraceptive Vaccine

GonaCon™ is a contraceptive vaccine that stimulates an immune response in a vaccinated animal by producing antibodies that bind to a gonadotropin-releasing hormone (GnRH). GnRH is a naturally occurring hormone that signals production of sex hormones such as estrogen, progesterone, and testosterone. The anti-GnRH antibodies interfere with the ability of GnRH to signal production of sex hormones, resulting in temporary infertility. As long as adequate levels of anti-GnRH antibodies are present in the vaccinated animal, sexual activity, breeding, and reproduction are extremely unlikely.

GonaCon™ is currently approved under the United States Environmental Protection Agency's (EPA's) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for use in female white-tailed deer as one tool to aid in reducing deer overpopulation (EPA Registration Number 56228-40). The immune response that causes temporary infertility in deer is accomplished with a single-shot vaccine. The length of time that a vaccinated female deer remains infertile depends on the individual animal, but some pen studies have shown that 4 out of 5 female deer remain infertile for 5 years (Miller et al., 2008a). Field studies have demonstrated lower rates of infertility ranging from 88% and 47% the first and second year after vaccination, respectively (Gionfriddo et al., 2009) to 67% and 43% the first and second year after vaccination, respectively (Gionfriddo et al., 2011a).

GonaCon™ is not currently registered for use in bison. However, USDA conducted a small pilot study of penned bison and found that none of the 6 females vaccinated with GonaCon™ became pregnant the first year after treatment (Miller et al., 2004). In 2011, APHIS received approval from EPA to use GonaCon™ in female bison in the confined experimental use scenario discussed in this EA. Should the proposed study discussed in this EA proceed, the data obtained from it could potentially be used to add to the required data set needed for EPA to register the GonaCon™ vaccine for use in bison. However, the purpose for registering GonaCon™ in bison would not be for reducing overpopulation. The intended purpose of using GonaCon™ in female bison would be to manage reproduction in bison known to be infected with brucellosis by inducing temporary infertility, thereby decreasing the potential for transmission of brucellosis through abortion and calving events.

B. Purpose of and Need for the Proposed Action

The purpose of the proposed action is to conduct a study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by preventing pregnancy, calving, and abortion, thereby preventing transmission of *B. abortus*. The major objectives of the proposed study are:

- To evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in *B. abortus*-infected female bison;
- To evaluate the effect on shedding by *B. abortus*-infected female bison that are rendered temporarily infertile by GonaCon™; and
- To evaluate the effect the infertility produced by GonaCon™ has on the long-term survivability of *B. abortus* in infected female bison.

Use of an effective immunocontraceptive such as GonaCon™ to prevent pregnancy and eliminate the potential for abortions by infected bison would break the cycle of transmission of brucellosis. If female bison known to be infected with *B. abortus* do not become pregnant, they would not abort. Exposure of non-infected animals to the infected tissues and fluids from aborted fetuses would therefore be reduced.

The need for the proposed study is to provide information that would be used to evaluate the use of GonaCon™ as a nonlethal method of decreasing or controlling the risk of transmission of *B. abortus* in the YNP bison population. Brucellosis is spread within the animal population primarily through contact with infected birthing tissues or aborted fetuses and through the milk of infected cows. If GonaCon™ can effectively render brucellosis-infected female bison temporarily infertile, the primary routes of disease transmission would be blocked. In combination with other appropriate disease mitigation activities, the use of GonaCon™ may be an effective tool to assist in eliminating brucellosis from the YNP bison herd over time.

USDA APHIS has determined that under the provisions of the National Environmental Policy Act (NEPA) (see 42 U.S.C. 4321 et seq.) and APHIS' National Environmental Policy Act (NEPA) implementing procedures (see 7 CFR Part 372), an EA should be prepared for these proposed actions. The availability of this EA and a 30-day comment period will be announced by publishing a notice on the APHIS brucellosis program website, the IBMP website and/or local newspapers. APHIS' decision maker for the actions described in this EA will take appropriate action after reviewing the EA, its associated analyses, public comments received, and other relevant responses and recommendations.

II. Proposed Action and Alternatives

A. No Action (the Current Situation)

The no action alternative would result in not conducting the proposed study. If the proposed study is not conducted, the utility of GonaCon™ as a non-lethal reproductive control option in bison cannot be determined. Additionally, if the use of GonaCon™ in bison is not investigated, information would not be known on whether temporary infertility induced by GonaCon™ is effective in decreasing the shedding of *B. abortus* and ultimately the transmission of brucellosis. Without the proposed study, use of the immunocontraception approach as a viable disease management tool for bison would not be evaluated, and could not be considered as a potential management tool.

B. Proposed Action

The proposed action is to conduct a multi-year study to evaluate the potential for use of GonaCon™, an immunocontraceptive vaccine, as a non-lethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy, thereby preventing abortions and risk of transmission of brucellosis to uninfected animals from contact with infected tissues and fluids from aborted fetuses.

The proposed study would include the following activities that are discussed in further detail below:

- Capturing bison in the late winter/spring of 2011, 2012, 2013, and possibly 2014;
- Transporting the captured bison by stock trailer to APHIS' bison facilities in Gardiner, Montana;
- Collecting and evaluating blood samples to determine brucellosis infection status at the beginning of the study and monitoring infection status at regular intervals throughout the study;
- Housing, caring for, and tagging (for identification purposes) animals in Gardiner, Montana facilities;
- Injecting one group of seropositive female bison with GonaCon™ beginning in the spring of 2012;
- Commingling uninfected bulls with females during breeding season, documenting breeding behavior, and testing for pregnancy for five calving seasons;
- Monitoring pregnant bison with transmitters and daily observing them for abortions, labor, and births;
- Collecting and testing blood, milk, and vaginal swabs from female bison that give birth to test for brucellosis infection status;

- Monitoring exposure to aborted fetuses by other bison and evaluating fetuses collected during the study; and
- Evaluating data collected from the study to determine whether GonaCon™ decreases the shedding of *B. abortus* in bison.

Bison for the proposed study would be acquired during the winter when they naturally exit YNP. The capture of bison would be conducted using methods currently in use for capturing bison according to the details of the IBMP operating procedures (IBMPOP, 2009). These procedures include hazing and/or using weed-free hay to move them to a capture facility. Approximately 104 adult bison would be used in the proposed study: 24 female bison that are seronegative for brucellosis; 72 female bison that test seropositive for brucellosis; and 8 male bison (bulls) that test seronegative for brucellosis. Female bison would be yearlings, two-, and three-years of age. If temporary chemical immobilization of any animal is needed, opioid narcotics and alpha-2-adrenergics would be used by study personnel qualified in the administration of such drugs. All bison used in the study would be identified with uniquely numbered ear tags and microchip identification.

The proposed study would take place on several double-fenced pastures at facilities in the Gardiner, Montana area: the Brogan Bison Facility in Corwin Springs (60 acres), the Slip 'n Slide pasture (25 acres), and the Rigler pasture (32 acres), all of which are located north of Gardiner, Montana. All sites are within the GYA and along Highway 89. The Brogan Bison Facility, Rigler pasture, and Slip 'n Slide pastures are currently leased by APHIS VS and Montana Fish, Wildlife and Parks and are used by APHIS VS for the bison quarantine feasibility study (MFWP, 2005). These facilities were specifically designed and erected to hold bison in a quarantine environment with hay and water as needed for an extended period of time.

The study design is as follows: In spring 2012, animals would be randomly selected to go into groups of 16 to 18 seropositive cows, four to six seronegative cows, and two bulls. Two replicate test pastures would be established in 2013 and possibly 2014 if not enough animals are captured in 2013. After three to four weeks of acclimation in the test pastures, *B. abortus*-infected female bison in one of the pastures would receive GonaCon™ vaccine (containing 3,000 micrograms in 3 milliliters of an adjuvant) delivered into the muscle on each side of the neck. The sites of injection would be tattooed and observed for any injection reaction. Bison in the remaining pasture would not be vaccinated.

Bulls would be separated from the cows outside of the breeding season from October to July. Prior to exposure to bulls, cows would have

breeding tags⁴ attached to them to document if bulls have mounted them to breed. Following first exposure of cows to bulls in 2012, five calving seasons would be observed (2013-2017). In February of each year, cows would be pregnancy-tested and fitted with vaginal transmitters to alert investigators to abortion or calving events.

During the abortion/calving seasons (from February until August of each year), daily observation for abortions, labor, and calving events would be conducted by study investigators. Within five days of abortion or calving, the cow would be immobilized and blood, milk, and vaginal swabs would be collected for testing. If possible, the calf would also be captured and eye swabs and blood would be collected for testing.

Following an abortion, the fetus would be left at the abortion site for 24 hours to monitor exposure to other bison. The fetus would then be collected, tested, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, Montana.

Blood testing of cows, bulls, and calves would be conducted three times a year: in February, calving time, and in the fall. Blood would be analyzed at the MVDL and/ or the National Veterinary Service Laboratories in Ames, Iowa throughout the study to determine *B. abortus* infection status of each animal.

Handling and physical restraint of bison for tagging or blood collection would take place in alleyways leading to standard bison manual squeeze chutes. Injection of the study animals with GonaCon™ would be done by study personnel experienced in administering intramuscular vaccines. Blood samples from study animals would be collected using established techniques for collection of blood from bison and would be performed by study personnel experienced with these techniques. An attending veterinarian would be available to address concerns about animal care and use for the study.

When the study is completed, all seropositive animals would be humanely euthanized following American Veterinary Medical Association-approved guidelines, and specimens would be collected from each animal for laboratory analysis. In addition, eggs and semen would be collected from these animals and frozen for genetic conservation. Per the conditions of the approval from EPA to use GonaCon™ in bison in this confined experimental use study, animals treated with GonaCon™ cannot be consumed by humans. These animals would be disposed of by incineration or landfill burial. Seropositive animals from the study that have not received GonaCon™ injections would be distributed to Montana food

⁴ Breeding tags are devices that are temporarily adhered to the base of the cow's tail that indicate by a color change that the cow has been mounted.

banks as is routinely done with other YNP seropositive bison. Further discussion on the safety of consuming bison infected with *B. abortus* is discussed in the human health and safety section of this document. All animals that test negative for brucellosis for the duration of the study and satisfy existing bison quarantine release requirements outlined in the APHIS Uniform Methods and Rules (USDA APHIS, 2003) would be used for bison conservation purposes.

Commented [mm1]: Can we say that we could use them as donor animals for genetic preservation in approved research facilities?

C. Other Alternatives Considered but Dismissed from Further Consideration

Because the most common route of transmission of *B. abortus* is contact with infected birthing fluids, aborted fetuses, and placental tissues, different methods of impacting the fertility of bison through the use of immunocontraceptive vaccines were considered as alternatives to the proposed action. If pregnancy could be prevented in *B. abortus*-infected female bison, transmission of *B. abortus* by this route could be eliminated or decreased.

APHIS considered the use of Porcine zona pellucida (PZP), another type of immunocontraceptive vaccine that has been used in animal species such as dogs, coyotes, burros, wild horses, and deer (USDA APHIS, 2010b). PZP has also been demonstrated to effectively induce temporary infertility in captive bison (Frank et al., 2005). However, research has shown that the use of PZP can increase the period of time in which the treated animals exhibit breeding season behavior.

The PZP vaccine results in temporary infertility while still allowing female animals to have multiple estrous cycles in which they engage in prebreeding behavior and breed. This behavior can cause animals to use energy at times of the year, such as late fall and early winter, when they would otherwise be conserving energy. Miller et al. (2004) concluded that "...Prolonging the breeding season of bison in the GYA may be deleterious to the winter survival of dominant bulls and PZP vaccinated cows because of increased sexual activity during fall and early winter." Therefore, this alternative was dismissed from further consideration because investigating the use of a PZP vaccine would not be useful in brucellosis management strategies in bison since it is associated with increased mating and reproductive activity (Killian et al., 2007).

APHIS also considered the alternative of physical sterilization as a means of decreasing the transmission of *B. abortus* within bison populations and between bison and cattle in the GYA. Physical sterilization such as

spaying⁵ or castration⁶ has been recognized as a disease management strategy that could be used to reduce the potential transmission of brucellosis in infected wildlife populations. However, this type of sterilization is permanent. APHIS would not subject the bison in the study to physical sterilization. For this reason, this alternative was dismissed from further consideration.

III. Potential Environmental Impacts

The NEPA implementing regulations provide criteria that Federal agencies should evaluate, if applicable, in environmental documents for proposed actions. This section of the EA addresses the applicable criteria related to potential impacts from the no action alternative and from the proposed action. NEPA criteria that are applicable for consideration in this section of the document include animal impacts, human health and safety, and the physical environment.

A. No Action

Without the proposed action, efforts to gather scientific information to better understand the potential application of immunocontraceptive vaccines such as GonaCon™ as a nonlethal strategy for reducing the transmission of *B. abortus* and decreasing the prevalence of brucellosis in the wild bison population in YNP would be lost. Without the proposed action to assist in developing nonlethal strategies to effectively control and eliminate brucellosis, the disease may continue to spread within the wild, free-ranging bison population in the GYA.

B. Proposed Action

a. Bison

The proposed study would not increase the risk of brucellosis being transmitted within the bison population. Therefore, this section focuses on the potential effects of the administration of GonaCon™ in bison.

In this proposed study, the desired effect of administering GonaCon™ is the temporary suspension of reproductive activity in the vaccinated female bison. Miller et al. (2004) report that “The gonadotropin-releasing hormone (GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns

⁵Surgical removal of the ovaries from female bison.

⁶ Surgical removal of the testes of male bison.

as the GnRH antibody titer drops below a protective level.” If the effect of this immunocontraceptive vaccine successfully places the vaccinated bison cows in a temporary nonreproductive state, the transmission of brucellosis by the female bison via shedding of *B. abortus* during calving or abortion may be eliminated.

A small study conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho in 2002-2003 demonstrated “that a single injection of GnRH vaccine is effective in preventing conception in female bison for at least 1 yr” (Miller et al., 2004). In that study, three of the six GnRH-treated bison cows and five of the untreated bison cows were in the last month of pregnancy at the time of vaccination. They delivered normal calves in the first year, suggesting that the GnRH vaccine did not interfere with the pregnancy and could be administered safely during the last third of the pregnancy. Additionally, none of the six treated bison became pregnant during the first breeding season (Miller et al., 2004).

Undesired health effects have been minimal in the species of wildlife in which GonaCon™ has been used. Injection site reactions caused by the “water-in-oil (W/O) emulsion needed in the GonaCon™ formulation for development of a long-term immune response” have been observed (Miller et al., 2008b). These reactions were most commonly manifested as inflammation or swelling at the injection site, or the presence of granulomas (thickened tissue filled with fluid). This observation is not uncommon in other livestock vaccines (USDA APHIS, 2010b).

As part of the GonaCon™ EPA registration process for use in deer, the health effects to the vaccinated deer were evaluated. Vaccinated animals showed no external evidence of inflammation at known injection sites; however, when muscle tissue at the injections site was sectioned, the injection sites appeared to be comprised of whiteish scar tissue, some containing vesicles of sterile fluid. All blood chemistry analyses were similar between treated and untreated deer. (Killian et al., 2006). Other types of injected products that alter animal hormones are currently used in livestock in the United States (USDA APHIS, 2010b).

Ensuring humane handling and treatment of all bison during the proposed study activities would be a priority. Application of animal identification tags, administration of GonaCon™ vaccine, and evaluation of pregnancy status, calving, or abortion activities would be conducted at appropriate times during the proposed study. These activities would be overseen by the study’s attending veterinarian and would not be expected to cause more than momentary or slight pain or discomfort. All temporary restraining and handling or temporary immobilization and handling activities would be conducted as quickly and efficiently as possible and in a manner that would prevent undue stress, trauma, injury, or any

unnecessary discomfort to the animal. If temporary immobilization is necessary, bison cows would be immobilized in locations within the facilities that are safe for the animals and the proposed study personnel. Veterinary oversight for animal care and handling, restraint, and sample collection would be provided during the proposed study activities. Wildlife biologists trained and experienced in the handling of bison would also be participating in the proposed study activities.

If necessary, study personnel would use the Federal Drug Administration (FDA)-approved anaesthetic and pain-killing (analgesic) drug combinations to immobilize the animals in order to prevent any potential negative impacts to the bison during the collection of study samples. The immobilization drugs would be used according to standard animal administration techniques, and it is expected that the bison would be immobilized for no more than 20 minutes. Vital signs of the immobilized bison would be monitored by qualified study staff throughout the sampling procedures and the initial recovery phase. To further ensure humane handling of the bison, every precaution would be taken by study staff to prevent immobilization- or handling-related trauma, injury, or death to the bison. The standard chemical immobilization protocol that would be used in this proposed study is widely used in bison and other wild ungulates without long-term effects (Kreeger et al., 2002).

In the GonaCon™ EPA registration process for use in deer, concerns were initially raised by some States that GonaCon™ would eliminate the need to use hunting as a tool to control deer overpopulation. Contraception alone would not reduce overabundant deer populations to healthy levels (USDA APHIS, 2010b). In deer, GonaCon™ is meant to be used in combination with other wildlife management tools to control populations. Assuming the use of GonaCon™ is eventually registered by EPA for bison, it is equally implausible to conclude that use of the contraceptive vaccine in bison would result in any significant population decreases in bison in the absence of other management tools (USDA APHIS, 2010b).

b. Non-Target Species

The proposed study would not increase the risk of brucellosis being transmitted to non-target species. Therefore, this section focuses on the risk of non-target species being exposed to GonaCon™.

In the proposed study, it is unlikely that non-target species would be exposed to GonaCon™. The proposed study protocol includes both risk mitigation measures that prevent direct exposure of non-target species to GonaCon™ and measures that limit the potential for secondary exposure of non-target species to GonaCon™.

To prevent direct exposure to non-target species, GonaCon™ would be administered directly to the study bison by hand-injection with a syringe. By using this direct-injection method, there would be no potential for accidental injection of non-target species with GonaCon™.

To prevent the risk of secondary exposure, the study plan includes measures to restrict access to treated animals by predators or other non-target species. To prevent access by larger wild animals, the bison in the proposed study would be maintained in double-fenced pastures, not on open range, thereby physically limiting potential contact between treated bison and wild animals such as elk, bears, and coyotes.

Abortions or calving events by GonaCon™-treated bison should be very minimal since the expected effect of treatment with GonaCon™ is to prevent pregnancy. The proposed study protocol includes actions to detect abortion or calving events, and the fencing would also physically limit some wild animals from accessing infected bison tissues from the GonaCon™-treated bison. The study protocol also includes standard operating procedures for proper removal and disposal of *B. abortus*-infected animal tissues from GonaCon™-treated bison from the study area to further limit potential exposure.

As discussed above, some larger animal species can be physically prevented from accessing the study area. However, some species such as birds of prey, smaller rodents, or insects cannot be prevented from accessing the study area. In the event that a non-target species were to consume GonaCon™-treated infected bison carcasses or GonaCon™-treated *B. abortus*-infected animal tissues, there would be no anticipated adverse effects from the GonaCon™ vaccine. Because GonaCon™ is made of proteins, it is broken down into smaller amino acids through digestion when it is consumed and has no contraceptive effect on non-target species (Fagerstone et al., 2008; Fagestone et al., 2010).

As part of the registration process for the use of GonaCon™ in deer, EPA concluded that there is no known danger associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007). Similar injectable hormone-altering products are used routinely in livestock applications (USDA APHIS, 2010b).

2. Human Health and Safety

a. General Public

The proposed study discussed in this EA would be conducted on double-fenced, private facilities where access by the general public to bison and potentially infected animal tissues such as aborted fetuses or reproductive materials would be prohibited. The protocol for the study contains standard operating procedures for handling and safely disposing of any

potentially brucellosis-infected materials generated from the animals in the study. The general public would have no risk of being exposed to either GonaCon™ -treated or untreated animals from the study or any potentially infected materials generated from the study.

There is no danger of transmission of the infection to humans from consuming cooked meat from *B. abortus*-infected bison. The *B. abortus* bacteria that causes brucellosis is typically not found in muscle tissue, and normal cooking temperatures kill any existing bacteria (USDA APHIS, 2011). Additionally, EPA and FDA concluded that there are no known human food safety concerns associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007 and FDA, 2005).

b. Worker Safety

Personnel who would be involved in the proposed study are qualified and have the expertise and experience needed to carry out the study activities. These activities include wildlife chemical immobilization, proficiency in administration of animal vaccines, veterinary care, animal restraint, tagging and marking animals, sample collection, and field evaluation of reproductive behaviors and activities.

Standard operating procedures would be in place to protect personnel involved in carrying out the proposed study activities. The standard operating procedures would include measures for safe and humane handling of bison to prevent injury to study personnel and to bison; safe handling and administration of GonaCon™; safe and humane collection of study samples for analysis; and safe handling procedures for study samples, including the safe handling and proper disposition of potentially infected animal tissues. In addition to the standard operating procedures and safety measures, at least one cell phone would be available at all times to facilitate contact in emergencies, and first aid kits would be available at all times in the event of injury to study personnel.

The GonaCon™ immunocontraceptive vaccine would be provided for the study in pre-mixed syringes and stored in locked containers except when actively being used to inject study animals. Personnel handling the vaccine would take appropriate precautions to prevent accidental self-injection. Pregnant women would not be involved in the handling or injecting of GonaCon™ at any time during the proposed study to avoid any potential risks associated with accidental exposure to the immunocontraceptive vaccine. Immobilization drugs and associated reversal drugs would be available for use if needed in the study. These drugs would be properly stored in locked containers to prevent improper access.

3. Physical Environment

As previously mentioned, proposed study activities would occur in several pastures at the Brogan Bison Facility, just north of Corwin Springs (60 acres), and the Slip ‘n Slide pasture (25 acres) and/or Rigler pasture (32 acres), located north of Gardiner, Montana.

The Brogan Bison Facility is used by APHIS VS for bison research. Forage at the pastures includes a mix of cultivated and native grasses. The upper pasture is on a steep slope along the west side of the property with several grass benchlands⁷ and steep, rocky drainages. The vegetation is composed of thinly forested slopes, interspersed with native bunchgrass rangelands (MFWP, 2005). Bassett Creek runs through the property and flows into the Yellowstone River.

The Slip ‘n Slide and Rigler pastures are located in close proximity to each other, just south of Yankee Jim Canyon. The pastures are double-fenced. The landscape is gently sloping and consists mostly of native grassland, except for the mixed alfalfa- and grass-cultivated hay meadows. Slip ‘n Slide Creek runs through the Slip ‘n Slide property and flows into the Yellowstone River. There are no brooks or creeks running through the Rigler pastures. The pastures are primarily surrounded by Gallatin National Forest and State of Montana land. Montana Fish, Wildlife and Parks historically leases the pastures on the ranch for bison to graze on (MFWP, 2011).

The potential environmental impacts of the proposed study on the physical environment would primarily be due to bison grazing in confined areas. The main issues of concern regarding confined grazing are effects on soil, vegetation, and water quality. These aspects are discussed below.

a. Soil and Vegetation

Livestock grazing in confined pastures can negatively affect soil quality by compacting the soil or causing soil erosion due to loss of vegetation cover. With a loss of vegetation, invasive species also threaten pastures. Most studies and discussions on the impacts of grazing focus on cattle because 70% of the western United States is grazed by livestock, which is primarily composed of cattle (Fleischner, 1994). Cattle are similar to bison in that they are large generalists and ungulate herbivores that can disturb terrestrial communities; however, differences in the two animals, such as forage selection and social organization (Hartnett et al., 1997; Steuter and Hidingen, 1999), may influence their impacts on soil and vegetation.

⁷ Steps or shelves in the mountainside that are the remains of former riverbanks or lakeshores.

Bison have a stronger preference for perennial grasses than cattle. Cattle consume a higher percentage of forbs⁸ in their diet than bison, and they use wooded areas and riparian zones more intensively than bison (Steuter and Hiding, 1999). Due to the lower diversity of plants consumed by bison and the bison's preference for herbaceous vegetation, there may be a reduction in the abundance of dominant grasses, an increase in the survival of subordinate species, and an increase in species diversity, when compared to land grazed by cattle (Hartnett et al., 1997). Additionally, physical disturbances that bison exhibit during non-grazing activities, such as wallowing⁹ may assist in ecodiversity (Hartnett et al., 1997).

The proposed action would not alter historic land use (for information regarding historic or cultural sites, see section below in the section on other environmental review requirements) at the pastures; therefore, overall effects to soil and vegetation would not be increased. Approximately 100 bison would be placed on 120 irrigated acres of land, averaging about one acre of land per bison. This density is expected to have only minimal impacts on the land. In addition, landowners at each ranch or facility implement management practices to minimize effects to soil and vegetation. Pasture rotation is practiced at or between facilities as necessary, so that each pasture is periodically rested and the land is not overused. Lastly, the bison at all facilities would be supplemented with hay, further limiting pasture grazing.

b. Water

GonaCon™ is a protein that is broken down within the treated bison; its metabolites would not be anticipated to be any greater than what would naturally occur. Therefore, this section focuses on other potential environmental impacts of bison grazing near water.

Potential environmental impacts from bison grazing in pastures could include a degradation of nearby water quality by manure, urine, and sediment being deposited into local waters. While bison that have access to a water body may directly deposit manure and urine into the water, wastes excreted onto land may also be transported to water bodies via leaching and surface runoff.

Grazing management practices can lessen the environmental impacts of streamside pastures. While many studies describe the impact of cattle grazing on water bodies, few studies have concentrated on the effects of native ungulates on stream health. Russell et al. (2009) states that the

⁸ Herbaceous flowering plants other than grass.

⁹ When bison roll in shallow depressions in the soil, covering themselves with dirt or mud.

proximity of cattle to the stream, the amount of time they spend by or in the stream, and the intensity and length of cattle grazing can all influence the water quality of nearby streams. One can assume the same behaviors in bison would also impact water quality.

Bison spend less time in streams or riparian habitats than cattle (Fleischner, 1994). Fleischner describes a study conducted in Utah regarding the feeding ecology of cattle and bison. The study noted that “cattle distribution was limited to gentle slopes near water, regardless of forage, while bison roamed widely, seemingly unaffected by slope or proximity to water.” As previously mentioned, cattle forage on a higher percentage of forbs and woody vegetation and maintain a larger breadth of diet niche than bison. Fritz et al. (1999) takes this one step further and states that a higher percentage of forbs and woody vegetation occurs in the riparian zone, so cattle are more likely to impact stream riparian zones than bison.

Fritz et al. (1999) studied the distribution and diversity of macroinvertebrates (e.g., insects, worms, snails and crayfish) in relation to bison crossings in prairie streams. The study suggests that impacts of bison on communities at the bottom of the streams was spatially limited, and that the bison may have less impact on stream communities than other studies of the impact of cattle. While comparison to cattle provides a noteworthy point of reference, it is important to point out that it is difficult to compare environmental responses with cattle versus bison due to confounding effects of site, weather, and management.

The pastures that would be utilized in the proposed study have historically been used for bison research or as livestock pastures, so deposits of manure, urine, and sediment due to the proposed study are not expected to increase the historic amount of contaminants entering the Yellowstone River. While the Brogan Bison Facility does have a creek running through it, bison do not have access to the creek. Only bison at the Slip ‘n Slide ranch would have direct, but limited, access to a creek. The access site to this creek was historically used for livestock and is at a point on the creek where the bank is shallow and covered with rocks. A shallow crossing means that bison would not have to climb up and down the bank, which would eventually cause the banks to erode. In addition, water would be provided to the bison, limiting the time that bison would visit the creek. Lastly, because only a portion of the total number of bison tested would be present on this pasture and those bison would spend limited time in streamside environments, the impact to water bodies is expected to be minimal.

IV. Other Environmental Review Requirements

A. Endangered or Threatened Species

Section 7 of the Endangered Species Act (ESA) and its implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of endangered or threatened species or result in the destruction or adverse modification of critical habitat. Proposed study activities would occur in pastures in southern Park County in Montana.

There are two federally listed mammals in Park County: the Canada lynx (*Lynx canadensis*) and the grizzly bear (*Ursos arctos horribilis*). Critical habitat has been designated for the Canada lynx in Park County.

Canada lynx: Areas designated as critical habitat for the Canada lynx include boreal forest landscapes that provide one or more of the following primary constituent elements for the lynx: snowshoe hares for prey; abundant, large, woody debris piles that are used as dens; and winter snow conditions that are generally deep and fluffy for extended periods of time (USDOI FWS, 2009).

Grizzly bear: In Montana, grizzly bears primarily use meadows, seeps, riparian zones, mixed shrub fields, closed timber, open timber, sidehill parks, snow chutes, and alpine slabrock habitats. Habitat use is highly variable between areas, seasons, local populations, and individuals. Grizzly recovery zones (areas identified where grizzly bear distribution is primarily within), including the Yellowstone area in northwest Wyoming, eastern Idaho, and southwest Montana (9,200 square miles), are estimated at more than 580 bears (FWS, 2011).

At all three locations, the pastures are double-fenced with an 8-foot woven wire fence and an electric high tensile fence to contain the study bison. These fences would also prevent Canada lynx and grizzly bears from entering the pastures. If Canada lynx or grizzly bears were to enter the pastures and consume GonaCon™-treated bison, there would be no effect on these species. The vaccine is made of proteins, and when consumed, is broken down into amino acids in the gastrointestinal tract, thereby having no contraceptive effect (Fagerstone et al., 2008; Fagerstone et al., 2010).

Federally-listed species and other non-target wildlife would not be directly exposed to GonaCon™ because the vaccine would be injected directly into the test bison and not administered orally in bait form. No wildlife habitat would be altered or disrupted by proposed study activities. No

helicopters would be used as part of this proposed study; therefore, no disturbance to wildlife in the surrounding area is expected. Although the study pastures occur within the designated critical habitat of the Canada lynx, the proposed study would have no effect on the primary constituent elements of that habitat and would not adversely modify it. Therefore, APHIS has determined that the proposed action would have no effect on the grizzly bear or Canada lynx.

B. Bald and Golden Eagle Protection Act

The Bald and Golden Eagle Protection Act (16 U.S.C. 668-668c) prohibits anyone, without a permit issued by the Secretary of the Interior, from "taking" bald eagles, including their parts, nests, or eggs. The Act provides criminal penalties for persons who "take, possess, sell, purchase, barter, offer to sell, purchase or barter, transport, export or import, at any time or any manner, any bald eagle ... [or any golden eagle], alive or dead, or any part, nest, or egg thereof." The Act defines "take" as "pursue, shoot, shoot at, poison, wound, kill, capture, trap, collect, molest or disturb."

There are no known bald eagle nests around the facilities; nesting areas are further down river (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.). However, golden eagle nests could be in the vicinity of the facilities, although specific nests are not known. Therefore, the proposed study is not expected to have any impact on nesting bald or golden eagles. In addition, activities occurring during the proposed study would not differ significantly from activities normally occurring at these pastures. "Eagles are unlikely to be disturbed by routine use of roads, homes, and other facilities where such use pre-dates the eagles' successful nesting activity in a given area. Therefore, in most cases ongoing existing uses may proceed with the same intensity with little risk of disturbing bald eagles" (FWS, 2007). If study personnel discover the presence of any bald or golden eagle nests in the area, this information would be reported to the Wildlife Program Manager at Gallatin National Forest.

Golden eagles have been observed flying over the Brogan Bison Facility (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.) and bald eagles could be flying in the area as well. The activities that would occur during the proposed study would not differ significantly from activities that normally occur in these pastures. Therefore, no disturbance of eagles would occur as a result of the proposed study; eagles in the area would be accustomed to these activities.

Although program personnel would remove daily any aborted calves or treated or non-treated bison that could die during the study, bald and golden eagles in the area could potentially consume these items. However, "[i]mmunocontraception vaccines provide few risks for

consumptive use of dosed wildlife; the antibodies that prevent reproduction are only one of millions of other antibodies present in animals, all of which are harmless to the organism that digests them, like any other proteinaceous food consisting of amino acids” (Fagerstone et al., 2010). Therefore, no eagles would be harmed if consumption of these items occurred.

C. Historic and Cultural Resources

In accordance with Section 106 of the National Historic Preservation Act of 1966 and its implementing regulations¹⁰, APHIS prepared a summary of the proposed project and submitted it to the Montana State Historic Preservation Office (SHPO) for consideration of potential impacts to historic resources. On November 28, 2011, APHIS received a letter of concurrence from the Montana SHPO agreeing that there were no findings of potential impacts to historic resources for the proposed study.

D. Tribal Consultation and Coordination

In accordance with Executive Order 13175: Consultation and Coordination with Indian Tribal Governments¹¹, APHIS has prepared a summary of the proposed project and provided it to 26 tribes who may have interests in YNP. In addition to the 26 identified tribes, APHIS also provided a summary of the project to all tribes located near YNP and in States adjacent to Montana who might potentially have interest in the project.

On December 19, 2011, APHIS held a conference by telephone with tribes to provide an opportunity to discuss the proposed project in more detail and discuss potential concerns that the tribes may have. Tribes that participated in the call showed an interest in the details of the project, and several requested additional information on the history of the GonaCon™ immunocontraceptive vaccine. APHIS agreed to provide background information to tribes. No tribes voiced any major concerns about the project.

APHIS will continue to conduct outreach to interested tribes and keep them updated on the activities associated with the project.

¹⁰ National Historic Preservation Act of 1966 (16 U.S.C. 470f) and implementing regulations (36 CFR §800).

¹¹ Executive Order 13175: Consultation and Coordination with Indian Tribal Governments (65 FR 67249, November 9, 2000).

V. Cumulative Impacts

This EA examines the activities associated with a proposed study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by effecting temporary infertility in bison cows and thereby preventing transmission of *B. abortus*. Activities associated with the proposed study are not expected to result in adverse cumulative effects.

In order to conduct the proposed study, approximately 96 female and 8 male bison that naturally exit YNP over the period of as many as three years would be housed at pasture locations in the Gardiner, Montana area. Some of the female animals in the study would be injected with GonaCon™, which would reduce the likelihood of pregnancy and delivery of offspring in the treated animals. Untreated females may give birth to offspring, which would increase the total number of animals associated with the study.

In August 2011, the National Park Service conducted an annual bison population estimate (NPS, 2011). According to the 2011 survey, the total bison population in YNP was estimated to be approximately 3,700 bison. This total was approximately 200 lower than the survey from the previous summer, but the decrease was “within the natural range of expectation for wild bison.”

Assuming the proposed study would result in approximately 104 bison being removed from the larger bison population of YNP, the effect of removing this number of bison over multiple years is well within the natural range of expectation for bison. This decrease in the numbers of bison in YNP is not anticipated to cause any cumulative negative effects to the overall bison population.

One of the goals of the IBMP is to manage temporal and spatial separation of bison and cattle to mitigate potential transmission of brucellosis. Currently, this is accomplished through hazing, capture, test and slaughter of seropositive animals, and vaccination of seronegative animals and a limited hunt in Montana. The proposed study may provide important information that would allow for re-evaluation and re-consideration of some of the current IBMP activities. This may result in impacts to future decision-making regarding protocols for bison habitat management, bison vaccination strategies, and bison hunt activities. IBMP activities that could be impacted include strategies to maintain appropriate bison population and distribution, should bison habitat be expanded.

VI. Agencies or Persons Contacted

U.S. Forest Service, Gallatin National Forest

Montana Fish, Wildlife and Parks

Montana State Historic Preservation Office, Montana Historical Society

USDA, Animal and Plant Health Inspection Service, Veterinary Services

USDA, Animal and Plant Health Inspection Service, Policy and Program Development, Environmental and Risk Analysis Services

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From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: RE: Bison GonaCon EA - Draft Final Document
Date: Thursday, January 19, 2012 11:00:39 AM

Look good to me.

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Thursday, January 19, 2012 10:01 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: Bison GonaCon EA - Draft Final Document
This is a little long but if you all can get a chance to skim thru it today, it would be good. It goes out tomorrow.

Jack

From: Stephens, Stephanie H - APHIS
Sent: Thursday, January 19, 2012 9:20 AM
To: Rhyan, Jack C - APHIS
Subject: Bison GonaCon EA - Draft Final Document
Hi Jack-Attached is the draft final EA for the GonaCon bison study in Montana. We're doing some final reference checks and an editorial review today.
The whole document will be ready to transmit to Ryan Clarke tomorrow for publication in Montana newspapers. If you have time to review the attached before tomorrow and you have any comments or concerns, please let me know.
I've spoken with Ryan and he will handle putting announcements in local newspapers and on the IBMP website. Deb Donch will arrange to get the EA posted on the VS brucellosis website. All comments will go to an e-mail address I've set up: eacommments2012@aphis.usda.gov.
We'll announce a 30-day comment period. I've also attached the draft legal notice, which is what will actually get published in newspapers.
Let me know if you have questions about any of this process.
Thanks,
Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: Bison GonaCon EA - Draft Final Document
Date: Thursday, January 19, 2012 11:46:33 AM

[Keeping them and making in vivo embryos...](#)

From: Nol, Pauline - APHIS
Sent: Thursday, January 19, 2012 11:45 AM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: Bison GonaCon EA - Draft Final Document

It looks good to me too. I did some really dinky editing (the doc is attached) and have two comments regarding maybe mentioning having an approved ACUC protocol and the other making sure that it is clear we will try to salvage genetics from both nonvaccinates and vaccinates if possible. The text does say that but it is not entirely clear.

Matt, are you proposing keeping some of those animals to collect embryos from, or just collecting semen and ovaries when they get euthanized?

P

From: McCollum, Matthew P - APHIS
Sent: Thursday, January 19, 2012 11:13 AM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: Bison GonaCon EA - Draft Final Document

[Looks good.](#)

[Just one comment about the disposition of seropositive animals and GnRH treated animals... If we are still in the game of embryo transfer, they could be valuable as donors.](#)

Matt

From: Rhyan, Jack C - APHIS
Sent: Thursday, January 19, 2012 10:01 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: Bison GonaCon EA - Draft Final Document

[This is a little long but if you all can get a chance to skim thru it today, it would be good. It goes out tomorrow.](#)

Jack

From: Stephens, Stephanie H - APHIS
Sent: Thursday, January 19, 2012 9:20 AM
To: Rhyan, Jack C - APHIS
Subject: Bison GonaCon EA - Draft Final Document

Hi Jack-Attached is the draft final EA for the GonaCon bison study in Montana. We're doing some final reference checks and an editorial review today.

The whole document will be ready to transmit to Ryan Clarke tomorrow for publication in Montana newspapers. If you have time to review the attached before tomorrow and you have any comments or concerns, please let me know.

I've spoken with Ryan and he will handle putting announcements in local newspapers and on the IBMP website. Deb Donch will arrange to get the EA posted on the VS brucellosis website. All comments will go to an e-mail address I've set up: ecomments2012@aphis.usda.gov.

We'll announce a 30-day comment period. I've also attached the draft legal notice, which is what will actually get published in newspapers.

Let me know if you have questions about any of this process.

Thanks,
Stephanie

Stephanie H. Stephens

USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: [Rhyan, Jack C - APHIS](#)
To: [Powers, Jenny](#); [Nol, Pauline - APHIS](#)
Cc: [margaret_wild@nps.gov](#); [Rick Wallen](#)
Subject: RE: Bison gonacon shedding study
Date: Monday, January 14, 2013 1:43:23 PM

Thanks, all.

Jack

From: Powers, Jenny [mailto:jenny_powers@nps.gov]
Sent: Monday, January 14, 2013 1:39 PM
To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Cc: [margaret_wild@nps.gov](#); Rick Wallen
Subject: Bison gonacon shedding study

Hi Pauline and Jack,

I had a chance to catch up with Margaret today and she is on board with separating the pregnant brucella seropositive cows from the GonaCon treated pasture. Rick and I had a chance to talk on Friday and we are both happy with it too. I'd love to have a look at the addendum once it is ready for IACUC submission. Thanks much for including us on the thought process and decision making.

Much appreciated!

Jenny

--

Jenny Powers, DVM, PhD
National Park Service
Wildlife Health Branch
1201 Oakridge Dr. #200
Fort Collins, CO 80525
(970) 267-2122 (office)
(b) (6) (cell)
(970) 225-3585 (fax)
jenny_powers@nps.gov

From: [Nol, Pauline - APHIS](#)
To: ["Powers, Jenny"](#)
Subject: RE: Bison gonacon shedding study
Date: Monday, January 14, 2013 2:56:00 PM

Awesome Jenny! Thanks!

From: Powers, Jenny [mailto:jenny_powers@nps.gov]
Sent: Monday, January 14, 2013 1:39 PM
To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Cc: margaret_wild@nps.gov; Rick Wallen
Subject: Bison gonacon shedding study

Hi Pauline and Jack,

I had a chance to catch up with Margaret today and she is on board with separating the pregnant brucella seropositive cows from the GonaCon treated pasture. Rick and I had a chance to talk on Friday and we are both happy with it too. I'd love to have a look at the addendum once it is ready for IACUC submission. Thanks much for including us on the thought process and decision making.

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(970) 225-3585 (fax)
jenny_powers@nps.gov

From: [Orahood, Darcy S - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Bison GonaCon
Date: Friday, November 04, 2011 4:52:26 PM

Pauline,

I loaded syringes for the bison study with GonaCon lot GCIV-11-2 which was made 7/1/2011 by Jeff Kemp. The following data is from the analysis of GnRH in this particular lot and was performed by chemistry on the dates indicated. The % GnRH represents what was detected in the sample relative to GnRH put into the vaccine. These numbers seem pretty typical and I believe they fall within the margins for QA through the EPA.

<u>Date sample aliquotted</u>	<u>Date analyzed</u>	<u>Result: % GnRH</u>
7/7/2011	8/10/2011	59.7
7/7/2011	8/17/2011	60.7
8/24/2011	8/25/2011	57.5
8/24/2011	8/25/2011	63.1

Please feel free to let me know if you have any questions.

Thank you,

Darcy Orahood

Biological Science Technician

USDA National Wildlife Research Center

4101 LaPorte Ave

Fort Collins, CO 80526

Phone (970) 266-6061

From: Nol, Pauline - APHIS
Sent: Thursday, October 27, 2011 1:37 PM
To: Orahood, Darcy S - APHIS; Rhyan, Jack C - APHIS
Cc: Miller, Lowell A - APHIS
Subject: RE: Bison GonaCon

Thanks Darcy. Yes, 10+ 3cc doses is what we need. I'm comfortable with the earlier analyses, although I would like to see them. But if it would help with the validation of the protocol, looking at consistency etc., I would not have any objection to doing it again.

Pauline

From: Orahood, Darcy S - APHIS
Sent: Thursday, October 27, 2011 12:10 PM
To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Cc: Miller, Lowell A - APHIS
Subject: Bison GonaCon

Hello,

I have identified a batch of GonaCon that is available for use in your upcoming bison study. It was manufactured in accordance with the specifications for the registered formula as your study protocol requires and it was sent up to chemistry for analysis of free GnRH. Does it need to be analyzed again at this time or are the other two sampling dates (7/7/11 and 8/24/11) sufficient for your purposes?

Also, I wanted to confirm that you need 10 (plus a few extra) x 3 cc doses.

I'll load the syringes at the beginning of next week so please let me know if this is correct and if

additional analysis by chemistry is necessary.

Thank you,

Darcy Orahood

Biological Science Technician

USDA National Wildlife Research Center

4101 LaPorte Ave

Fort Collins, CO 80526

Phone (970) 266-6061

From: [Robbe Austerman, Suelee - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Wyckoff, Brenda K - APHIS](#); [Weese, Jacilyn R - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: Bison preg testing
Date: Tuesday, November 19, 2013 5:02:06 PM

Ahh, Jack... you were requesting information on how to order? Sorry I was at training all day. You are lucky you are not in Ames this week ☺

If your purchase will be under 3,000 you can use the laboratory that you choose. Please send to Jaci the following:

Vendor- name, address and phone number
What you are requesting and the cost per item
Jaci will reply back with a requisition number.

Please enter the requisition number on the submission form so that number ends up on the bill.

Forward the bill you get to us and we will make sure it gets paid.

We will have detailed work instructions for all purchases, just need to get time to write it. I am working on getting a CC for you all for local purchases.

From: Rhyan, Jack C - APHIS
Sent: Tuesday, November 19, 2013 4:17 PM
To: Wyckoff, Brenda K - APHIS
Cc: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Robbe Austerman, Suelee - APHIS
Subject: RE: Bison preg testing

Brenda,

Thanks so much for working on this. The Pregnancy Specific Protein B (PSPB) test is a well-established pregnancy test run on blood and used extensively by wildlife folks. It is offered by one lab in Moscow, Idaho (Biotracking). We have used them for years for this test and it's results correlate well with rectal palpation and ultrasound which we have also used for years. It's results are widely trusted among wildlife folks. In some situations with wildlife, a blood test is the only option to determine pregnancy. We used to just give Biotracking a credit card over the phone. Alternatively we received a bill and forwarded that for payment. It will be important for us to establish a way to continue doing business with them if at all possible.

Again, thanks very much for your help on this and many more issues yet to come, I'm sure.

Jack

From: McCollum, Matthew P - APHIS
Sent: Tuesday, November 19, 2013 2:57 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: Bison preg testing

Here is the answer I got...

Matt

From: Wyckoff, Brenda K - APHIS
Sent: Tuesday, November 19, 2013 10:31 AM
To: McCollum, Matthew P - APHIS
Subject: Bison preg testing

Hi Matt, I called around here as I said I would and below are the answers that I received. Please let me know if you need me to try to get some more info for you.

We don't use a serum test but rather use palpation and ultrasound.. It would be based on pregnancy specific protein B. A quick google search showed a test called BioPRYN is out there and also Linscott's directory of immunological and biological reagents has a bovine ELISA kit available. I suspect there is probably some other companies that offer testing kits or do testing. ~ Dr. Steven Olsen

Tell Matt that he might want to check with The Dairy Authority located in Greeley CO. They offer both the IDEXX and the Biopryn (sp) elisa. I suspect they will work just fine. ~ Dr. Suelee Robbe-Austerman

Thank you
Brenda Wyckoff
515-337-7565

From: [Rhyan, Jack C - APHIS](#)
To: [Wyckoff, Brenda K - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Robbe Austerman, Suelee - APHIS](#)
Subject: RE: Bison preg testing
Date: Tuesday, November 19, 2013 3:16:31 PM

Brenda,

Thanks so much for working on this. The Pregnancy Specific Protein B (PSPB) test is a well-established pregnancy test run on blood and used extensively by wildlife folks. It is offered by one lab in Moscow, Idaho (Biotracking). We have used them for years for this test and it's results correlate well with rectal palpation and ultrasound which we have also used for years. It's results are widely trusted among wildlife folks. In some situations with wildlife, a blood test is the only option to determine pregnancy. We used to just give Biotracking a credit card over the phone. Alternatively we received a bill and forwarded that for payment. It will be important for us to establish a way to continue doing business with them if at all possible.

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Sent: Tuesday, November 19, 2013 2:57 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: Bison preg testing

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515-337-7565

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To: [Robbe Austerman, Suelee - APHIS](#); [Rhyan, Jack C - APHIS](#); [Wyckoff, Brenda K - APHIS](#); [Weese, Jacilyn R - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: Bison preg testing
Date: Thursday, November 21, 2013 9:59:22 AM

Thanks Suelee and Brenda,

Sorry about this, I did not do a very good job conveying what I was asking for... I'll email Jaci the necessary info.

We'll learn the ropes.

Thanks for your patience,
Matt

From: Robbe Austerman, Suelee - APHIS
Sent: Tuesday, November 19, 2013 5:02 PM
To: Rhyan, Jack C - APHIS; Wyckoff, Brenda K - APHIS; Weese, Jacilyn R - APHIS
Cc: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: Bison preg testing

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Vendor- name, address and phone number
What you are requesting and the cost per item
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Thank you
Brenda Wyckoff
515-337-7565

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: bison proj
Date: Wednesday, May 11, 2011 2:25:00 PM

Probably a good way to do it logistically anyway.....with retaining the Slip N' Slide pasture up in the air. If we know exactly what number of animals we need to take off the traps going into next January then it's easier to get all the YNP personnel to work towards getting us those animals for us.

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell

☐ Jack C Rhyan---05/11/2011 11:52:12 AM---All, Jenny had good advice and I called Brant Schumaker to consult on the study design. His thought

From: Jack C Rhyan/CO/APHIS/USDA@MSOCOEX
To: Pauline Nol/CO/APHIS/USDA, Matt McCollum/CO/APHIS/USDA, Rebecca K Frey/MT/APHIS/USDA, Patrick R Clarke/MT/APHIS/USDA, "rick_wallen@nps.gov" <rick_wallen@nps.gov>, "margaret_wild@nps.gov" <margaret_wild@nps.gov>, "Jenny_powers@nps.gov" <Jenny_powers@nps.gov>
Date: 05/11/2011 11:52 AM
Subject: bison proj

All,
Jenny had good advice and I called Brant Schumaker to consult on the study design. His thought was to focus on the individual animals and shedding and use a few sentinels only as proof of concept. I think that makes sense so we will try to find 16 to 18 seropositives per pasture next year and only use about 4 sentinels per pasture. We may not be able to find enough animals by next year so we will write it such that we can do staggered starts. That is next year we at least start with one control and one vaccinate pastures. Then if need be, we can start the other 2 pastures the following year. Brant said that would not impact the stats. What do you all think?
Wagged by stats,
Jack

From: [Nol, Pauline \(APHIS\)](#)
To: Jenny_Powers@nps.gov; [Rhyan, Jack C \(APHIS\)](#)
Subject: RE: bison proj
Date: Wednesday, May 18, 2011 1:40:00 PM

Hi Jenny,
Yes, they are null hypotheses, not a reflection of my bad attitude!
P

-----Original Message-----

From: Jenny_Powers@nps.gov [mailto:Jenny_Powers@nps.gov]
Sent: Wednesday, May 18, 2011 12:12 PM
To: Rhyan, Jack C (APHIS); Nol, Pauline (APHIS)
Subject: Re: bison proj

Hi Jack and Pauline,

Are these the null hypotheses or what we actually think will happen? I must be missing something..... seems like we are suggesting that contraception will eliminate or at least decrease shedding.

(Embedded image moved to file: pic17148.jpg)

"Rhyan, Jack C
(APHIS)"
<Jack.C.Rhyan@aphis.usda.gov> To
, "McCollum, Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
05/11/2011 11:52 "Frey, Rebecca K (APHIS)"
AM <Rebecca.k.frey@aphis.usda.gov>,
"Clarke, Ryan P. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"rick_wallen@nps.gov"
<rick_wallen@nps.gov>,
"margaret_wild@nps.gov"
<margaret_wild@nps.gov>,
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<Jenny_powers@nps.gov>
cc

Subject
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Jack

From: Jenny_Powers@nps.gov
To: [Nol, Pauline \(APHIS\)](#)
Subject: RE: bison proj
Date: Wednesday, May 18, 2011 2:47:28 PM

Gotcha. Thanks. I haven't seen them written that way before in a proposal. Sorry for my denseness.

J

"Nol, Pauline
(APHIS)"
<Pauline.Nol@aphis.usda.gov> To
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>, "Rhyan,
05/18/2011 01:40 Jack C (APHIS)"
PM <Jack.C.Rhyan@aphis.usda.gov>
cc

Subject
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05/11/2011 11:52 "Frey, Rebecca K (APHIS)"
AM <Rebecca.k.frey@aphis.usda.gov>,
"Clarke, Ryan P. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"rick_wallen@nps.gov"
<rick_wallen@nps.gov>,
"margaret_wild@nps.gov"
<margaret_wild@nps.gov>,
"Jenny_powers@nps.gov"
<Jenny_powers@nps.gov>
cc

Subject
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Wagged by stats,
Jack

From: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
To: [Rhyan, Jack C \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: Re: bison proj
Date: Wednesday, May 18, 2011 12:13:44 PM

Hi Jack and Pauline,

Are these the null hypotheses or what we actually think will happen? I must be missing something..... seems like we are suggesting that contraception will eliminate or at least decrease shedding.

(Embedded image moved to file: pic17148.jpg)

"Rhyan, Jack C
(APHIS)"
<Jack.C.Rhyan@aphis.usda.gov> To
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<Matt.McCollum@aphis.usda.gov>,
05/11/2011 11:52 "Frey, Rebecca K (APHIS)"
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<margaret_wild@nps.gov>,
"Jenny_powers@nps.gov"
<Jenny_powers@nps.gov>
cc

Subject

bison proj

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Wagged by stats,
Jack

From: [Clarke, Patrick R. - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Thompson, Brent D - APHIS](#)
Subject: RE: bison roundup
Date: Friday, January 30, 2015 2:15:06 PM

And I'm not even that good at pushing papers,....but I've got another week at Plum and then a brucellosis review the week after that.

Not sure how much of the VOC gear we kept here in MT, if any.....Becky? We are set logistically for sampling in the slaughterhouses this year as far as permits, notifications, and equipment.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Nol, Pauline - APHIS
Sent: Friday, January 30, 2015 10:20 AM
To: Frey, Rebecca K - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Thompson, Brent D - APHIS
Subject: RE: bison roundup

Okay, we are trying to figure out logistics etc. In the meantime, I'm sending the ACUC amendment so that Clarke et al. can look at it...since all he is good for right now is pushing papers;)

Thanks!
Pauline

From: Frey, Rebecca K - APHIS
Sent: Friday, January 30, 2015 10:14 AM
To: Nol, Pauline - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Thompson, Brent D - APHIS
Subject: RE: bison roundup

No slaughter collection by the park this year. Just me and Brent right now, until the 13th really, as Clarke can't be around livestock and has other plans until the 13th. You would have to send re-enforcements to get it all done.

From: Nol, Pauline - APHIS
Sent: Friday, January 30, 2015 9:59 AM
To: Frey, Rebecca K - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: bison roundup

Sooooooooooooo, any chance we could do some breath collections again in the next few weeks? What's the status on able bodies up there? Do you know if the Park is doing any tissue collections again?

Pauline

From: Frey, Rebecca K - APHIS
Sent: Thursday, January 29, 2015 10:32 AM
To: Nol, Pauline - APHIS
Subject: RE: bison roundup

They want to ship another 5-600. Have trap active now, just nothing in right now. Probably start up again next week, they hope to be done in about 2 weeks though.

From: Nol, Pauline - APHIS
Sent: Thursday, January 29, 2015 10:23 AM
To: Frey, Rebecca K - APHIS
Subject: bison roundup

Hey Becky,

We are calling Israel today to talk to them about future research. Collecting buffalo breath for VOC's has come up again. How many more animals do they plan on trapping and sending off this year? Or are they near the quota?

Thanks and hope the day is going well!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Thompson, Brent D - APHIS](#)
Subject: RE: bison roundup
Date: Friday, January 30, 2015 10:13:47 AM

No slaughter collection by the park this year. Just me and Brent right now, until the 13th really, as Clarke can't be around livestock and has other plans until the 13th. You would have to send re-enforcements to get it all done.

From: Nol, Pauline - APHIS
Sent: Friday, January 30, 2015 9:59 AM
To: Frey, Rebecca K - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: bison roundup

Sooooooooooooo, any chance we could do some breath collections again in the next few weeks? What's the status on able bodies up there? Do you know if the Park is doing any tissue collections again?
Pauline

From: Frey, Rebecca K - APHIS
Sent: Thursday, January 29, 2015 10:32 AM
To: Nol, Pauline - APHIS
Subject: RE: bison roundup

They want to ship another 5-600. Have trap active now, just nothing in right now. Probably start up again next week, they hope to be done in about 2 weeks though.

From: Nol, Pauline - APHIS
Sent: Thursday, January 29, 2015 10:23 AM
To: Frey, Rebecca K - APHIS
Subject: bison roundup

Hey Becky,
We are calling Israel today to talk to them about future research. Collecting buffalo breath for VOC's has come up again. How many more animals do they plan on trapping and sending off this year? Or are they near the quota?
Thanks and hope the day is going well!
Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)

Fax: 970-266-6157

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: bison roundup
Date: Thursday, January 29, 2015 10:31:39 AM

They want to ship another 5-600. Have trap active now, just nothing in right now. Probably start up again next week, they hope to be done in about 2 weeks though.

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Sent: Thursday, January 29, 2015 10:23 AM
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From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Thompson, Brent D - APHIS](#)
Subject: RE: bison roundup
Date: Friday, January 30, 2015 2:33:52 PM

Pretty sure it is all back in CO. Unless it is at the storage unit, I don't have any.

From: Clarke, Patrick R. - APHIS
Sent: Friday, January 30, 2015 2:15 PM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Thompson, Brent D - APHIS
Subject: RE: bison roundup

And I'm not even that good at pushing papers,....but I've got another week at Plum and then a brucellosis review the week after that.

Not sure how much of the VOC gear we kept here in MT, if any.....Becky? We are set logistically for sampling in the slaughterhouses this year as far as permits, notifications, and equipment.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Nol, Pauline - APHIS
Sent: Friday, January 30, 2015 10:20 AM
To: Frey, Rebecca K - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Thompson, Brent D - APHIS
Subject: RE: bison roundup

Okay, we are trying to figure out logistics etc. In the meantime, I'm sending the ACUC amendment so that Clarke et al. can look at it...since all he is good for right now is pushing papers;)

Thanks!
Pauline

From: Frey, Rebecca K - APHIS
Sent: Friday, January 30, 2015 10:14 AM
To: Nol, Pauline - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Thompson, Brent D - APHIS
Subject: RE: bison roundup

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Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Rhyan, Jack C - APHIS](#)
To: [Becky Wills](#) [Becky Wills](#) [\(b\) \(6\)@uwyo.edu](#); [Rebecca E. Ashley](#)
Cc: [Dufficy, Deborah L - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: bison serum
Date: Friday, March 09, 2012 9:33:56 AM

Greetings Becky and Rebecca. We have about 15 seropositive bison in Fort Collins. The next time we work them we will be glad to get serum for you. I'm not sure when that will be but probably in the next month or 2. How would you like us to collect it or would you want to come down and transport it yourself?

Jack Rhyan

From: Dufficy, Deborah L - APHIS
Sent: Thursday, March 08, 2012 9:17 AM
To: Becky Wills [Becky Wills](#) [\(b\) \(6\)@uwyo.edu](#); [Rebecca E. Ashley](#)
Cc: Rhyan, Jack C - APHIS
Subject: bison serum

Dr. Rhyan will get back to you on brucellosis-positive serum from the donated bison. He thinks he can help out.

Deborah L. Dufficy, DVM, MPH, DACVPM
Wyoming Area Epidemiologist
USDA-APHIS-Veterinary Services
5353 Yellowstone Road, Suite 209
Cheyenne, WY 82009
office 307-432-7968
fax 307-772-2592

From: [Rebecca A. Wills](#)
To: [Rhyan, Jack C - APHIS](#); [Rebecca E. Ashley](#)
Cc: [Dufficy, Deborah L - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: bison serum
Date: Friday, March 09, 2012 10:43:12 AM


Dr. Rhyan, thanks so much for letting us in on this serum. We are not in any hurry and please only do this at your convenience. If you need me to send blood collection tubes and return boxes I can do that too. We can also test this serum for B. abortus antibodies (RAP and FPA) and give you our results if that in any way helps with your research. I can probably provide you with our UPS account number for shipping too. We ship positive blood samples so I don't think this should be a problem. Please just let me know what you need from me, and once again thank everyone for sharing and helping us out with this. Becky

Rebecca A. Wills
Wyoming State Veterinary Laboratory
Regulatory Serology
1174 Snowy Range Road
Laramie, Wyoming 82070
 [@uwyo.edu](mailto:(b) (6)@uwyo.edu)
(307) 766-9924
(307) 766-9917

From: Rhyan, Jack C - APHIS [mailto:Jack.C.Rhyan@aphis.usda.gov]
Sent: Friday, March 09, 2012 9:34 AM
To: Rebecca A. Wills; Rebecca E. Ashley
Cc: Dufficy, Deborah L - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: bison serum

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Sent: Thursday, March 08, 2012 9:17 AM
To: Becky Wills Becky Wills  [@uwyo.edu](mailto:(b) (6)@uwyo.edu); Rebecca E. Ashley
Cc: Rhyan, Jack C - APHIS
Subject: bison serum

Dr. Rhyan will get back to you on brucellosis-positive serum from the donated bison. He thinks he can help out.

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From: [Keith Roehr - CDA](#)
To: [Rhyan, Jack C - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); carl.heckendorf@state.co.us; [Linfield, Thomas F - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: bison ship on 127
Date: Wednesday, June 17, 2015 3:58:36 PM

Under the circumstances I agree, thanks.

Sent from my iPad

On Jun 17, 2015, at 3:35 PM, Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov> wrote:

Becky,

I visited with Dr. Heckendorf and explained the paucity of accredited vets in the area due to the bird flu wars. He thought it would be fine to ship on the 127 and an import permit.

Jack

From: [Rhyan, Jack C - APHIS](#)
To: keith.roehr@state.co.us
Cc: [Frey, Rebecca K - APHIS](#); carl.heckendorf@state.co.us; [Linfield, Thomas F - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: bison ship on 127
Date: Wednesday, June 17, 2015 4:02:44 PM

Thanks, Keith.

Jack

From: Keith Roehr - CDA [mailto:keith.roehr@state.co.us]
Sent: Wednesday, June 17, 2015 3:59 PM
To: Rhyan, Jack C - APHIS
Cc: Frey, Rebecca K - APHIS; carl.heckendorf@state.co.us; Linfield, Thomas F - APHIS; Nol, Pauline - APHIS
Subject: Re: bison ship on 127

Under the circumstances I agree, thanks.

Sent from my iPad

On Jun 17, 2015, at 3:35 PM, Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov> wrote:

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I visited with Dr. Heckendorf and explained the paucity of accredited vets in the area due to the bird flu wars. He thought it would be fine to ship on the 127 and an import permit.

Jack

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [keith.roehr@state.co.us](#); [carl.heckendorf@state.co.us](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: Re: Bison shipment 6.30.15
Date: Tuesday, June 30, 2015 6:12:59 PM
Attachments: [image1.JPG](#)

Bison arrived at Fort Collins facility safe and sound.

Thanks,

Matt 

Sent from my iPhone

On Jun 30, 2015, at 9:16 AM, Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov> wrote:

Hi Keith and Carl,
I'm attaching the paperwork (127 and test charts) for the bison that are shipping Sent from my iPhone
Thank you!
Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1-970-266-6126
Mobile: (b) (6)
Fax: +1-970-266-6157

<test chart bison(2) 6.30.15.pdf>

<test chart bison 6.30.15.pdf>

<127 Bison 6.30.15.jpg>

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES

PERMIT FOR MOVEMENT OF RESTRICTED ANIMALS

USE A SEPARATE FORM FOR EACH SPECIES

FORM APPROVED
OMB NO. 0579-0051

No. F101922

1. NAME AND ADDRESS OF SHIPPER OR CONSIGNOR (Include Zip Code)

USDA APHIS VS

GonaCom Study

Cowin Spring MT

2. CONSIGNEE (Destination Name and Address, include Zip Code)

USDA APHIS VS @NWRC

4101 LaPorte Ave

Fort Collins CO

5. STATE WHERE ISSUED

MT

6. MOVEMENT TO BE

☒ INTERSTATE ☐ INTRASTATE

7. MOVEMENT FOR

☒ QUARANTINE ☐ SLAUGHTER

8. DISEASE

Bruce/osis

9. STATUS OF ANIMALS

No. Reactor

4

No. Exposed

4

No. Other (Specify)

CO Permit #

1P00CNGR

10. STATUS OF HERD OF ORIGIN

Infected

11. STATUS OF AREA OF ORIGIN

DSA

3. MOVED FROM (Name and Location of Premise if other than item 1 above)

12. NO. ANIMALS IN THIS SHIPMENT

8

13. SPECIES (One only)

Bison

4. NAME AND ADDRESS OF OWNER AT TIME CONDITION DIAGNOSED

USDA APHIS VS

Cowin Spring MT

14. TRANSPORTATION VEHICLE LICENSE NO. OR OTHER IDENTIFICATION NO.

A372561 A362709

15. SEAL NO.

APHIS Hauling
3084271

16. VEHICLE REQUIRED TO BE CLEANED AND DISINFECTED AT DESTINATION

☐ YES

☒ NO

(If Yes, Items 32, 33, and 34 are Applicable)

VALID ONLY FOR ABOVE DESTINATION

17. ANIMALS TO BE MOVED

COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)	COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)
4618	Bis	F	N/A	840003003344153					
4R6		F		840003003344155					
4R13		F		840003003344154					
4R22		F		840003003344159					
3603		F		840003003344149					
4602		M		840003003344120					
4604		M		840003003344118					
4617		F		840003003344119					

I certify that I have inspected the animals described on this permit and find them eligible to move in accordance with the requirements of State and Federal regulations.

18. SIGNATURE OF INSPECTOR

19. DATE ISSUED

6/30/15

20. TIME ISSUED

7:00 AM

VOID AFTER

21. DATE

7/1/15

22. TIME

12:00 AM

WARNING TO OWNER, SHIPPER AND TRUCKER - LIVESTOCK MUST BE DELIVERED TO CONSIGNEE WITHOUT DIVERSION

I understand that it is a violation of Federal law to move the animals identified herein interstate except in accordance with the provisions of applicable Federal Regulations. I also understand that such animals must comply with existing state laws and regulations governing movement of livestock and poultry. I have arranged or will arrange for a copy of this permit to accompany the interstate shipment and be delivered with the above described animals.

23. SIGNATURE OF OWNER OF SHIPPER

(b) (6)

24. TITLE

☐ OWNER

☒ SHIPPER

25. DATE SIGNED

6/30/2015

I certify that the animals described on this permit were received and slaughtered/quarantined in accordance with the requirements of the State and Federal regulations on the date indicated in item 29.

26. PLACE ANIMALS RECEIVED

USDA APHIS VS Bison Peris

27. DATE ANIMALS ARRIVED

6/30/2015

28. NO. ANIMALS RECEIVED

8

29. DATE SLAUGHTERED/QUARANTINED

30. DATE AND TIME SEALS BROKE

6/30/2015

31. AUTHORIZED SIGNATURE

(b) (6)

32. DATE CLEANED AND DISINFECTED (if required)

33. SIGNATURE OF INSPECTOR


34. DATE SIGNED

From: [John D. Eisemann/CO/APHIS/USDA](#)
To: [Jack C Rhyan](#)
Cc: [Pauline Nol](#)
Subject: Re: Bison Study protocol
Date: Friday, January 21, 2011 9:38:00 AM

OK. Pauline, I am more than willing to help seeing as how this will be a EPA regulated study.


John Eisemann
USDA APHIS Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80526

T: 970-266-6158
F: 970-266-6157

 Jack C Rhyan---01/21/2011 09:21:14 AM---I figured. We'll get after it. Pauline is magic on these. She is out til next week. Jack

**Jack C
Rhyan/CO/APHIS/USDA**

01/21/2011 09:21 AM

To: John D Eisemann/CO/APHIS/USDA@USDA
cc: Pauline Nol/CO/APHIS/USDA@USDA
Subject: Re: Bison Study protocol 

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**John D
Eisemann/CO/APHIS/USDA**

01/21/2011 08:30 AM

To: Jack C Rhyan/CO/APHIS/USDA@USDA,
Stephanie H
Stephens/MD/APHIS/USDA@USDA,
Lowell A
Miller/CO/APHIS/USDA@USDA
cc

Subject: Bison Study protocol

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From: [Jack C Rhyan](#)
To: [John D Eisemann/CO/APHIS/USDA](#)
Cc: [Pauline Nol](#)
Subject: Re: Bison Study protocol
Date: Friday, January 21, 2011 9:41:00 AM


We'll definitely need your help!
Jack

☐ John D Eisemann---01/21/2011 09:38:57 AM---OK. Pauline, I am more than willing to help seeing as how this will be a EPA regulated study. John Eisemann

**John D
Eisemann/CO/APHIS/USDA**

ToJack C Rhyan/CO/APHIS/USDA@USDA
ccPauline Nol/CO/APHIS/USDA@USDA

01/21/2011 09:38 AM

SubjectRe: Bison Study protocol 

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ccPauline Nol/CO/APHIS/USDA@USDA

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Fort Collins, CO 80526

T: 970-266-6158

F: 970-266-6157

From: [Jack C Rhyan](#)
To: Jenny_Powers@nps.gov; [Pauline Nol](#)
Subject: RE: bison study
Date: Tuesday, May 10, 2011 4:50:00 PM

Jenny,

Good idea. I talked with Brant this afternoon and he is running some numbers for us. His off the cuff idea was concentrate on individual animal shedding and use just a few sentinels for proof of concept about transmission. I like that. I'll let you know what we arrive at for numbers.

Jack

-----Original Message-----

From: Jenny_Powers@nps.gov [mailto:Jenny_Powers@nps.gov]
Sent: Tuesday, May 10, 2011 1:11 PM
To: Rhyan, Jack C (APHIS); Nol, Pauline (APHIS)
Subject: bison study

Hi Jack and Pauline,

Certainly didn't mean to be a downer today on the phone call. I think its going to be an interesting study. I am having a hard time getting past the likelihood that transmission events aren't going to be independent within a pen and that the pen is the sampling unit. Maybe my argument doesn't hold water at all but I think it does highlight the need for someone with good study design skills to put some of our questions to rest. I'm wondering if you've had any change of heart on asking Brant Shumacher to be involved.

He seems like a good brain to think this one through....

After this week I'd be happy to put more time and thought into this. Sorry for my absence last week.

See you soon,
Jenny

From: [Keith Roehr - CDA](#)
To: [Nol, Pauline - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: Re: bison to be shipped soon from MT to Fort Collins
Date: Monday, June 15, 2015 4:26:53 PM

Thanks Pauline, please send the CVI and the VS 1-27 before the bison ship.

Thanks,

Keith

Sent from my iPad

On Jun 15, 2015, at 11:41 AM, Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov> wrote:

Hi Keith,

I wanted to give you a heads up that we are planning on bringing down up to 8 more bison from our facility in Gardiner, MT to the Fort Collins Facility. We don't know the date of transport yet (in the next 30 days) but you will be getting a Cert. of Inspection soon and test results etc. are pending. They will be travelling on a 1-27.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1-970-266-6126
Mobile: (b) (6)
Fax: +1-970-266-6157

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: RE: Bison to CO - only VS 1-27 required by CO
Date: Wednesday, June 17, 2015 9:18:27 AM

Yes it should be but now we have Keith expecting a CVI since we said we would send one. And he is apparently out of the office. I guess we have a few days since we are looking at the 30th to do this, but I wish everybody would just get on the same page, I don't want somebody getting mad because they aren't communicating in CO.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Wednesday, June 17, 2015 9:14 AM
To: Frey, Rebecca K - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: Re: Bison to CO - only VS 1-27 required by CO

I think the logic is that VS gives the state office a hard time about not having a CVI with shipments. If Dr Heckendorf is fine without the CVI then that should be good.

Sent from my iPhone

On Jun 17, 2015, at 07:03, Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov> wrote:

Could one of you provide any more information for Tom? Thanks to AI, nobody in MT for a health.

Sent from my iPhone

Begin forwarded message:

From: "Linfield, Thomas F - APHIS" <Thomas.F.Linfield@aphis.usda.gov>
Date: June 16, 2015 at 9:20:23 PM MDT
To: "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>
Cc: "Bailey, Glen R - APHIS" <Glen.R.Bailey@aphis.usda.gov>, "Thompson, Brent D - APHIS" <Brent.D.Thompson@aphis.usda.gov>, "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>
Subject: Re: Bison to CO - only VS 1-27 required by CO

The question arose today, primarily because Glen is not deputized in m MT / does not have any MT DOL issued CVI's. We tried calling Dr Roehr to confirm - he was out of town, however Dr Heckendorf acting in his absence authorized VS 1-27 only, along with obtaining a CO permit. He actually had concerns regarding issuing a CVI with the "statements of health/ lack of exposure to infectious, contagious diseases on bison known to be infected or exposed to Brucella. ...

Did Dr Roehr provide any logical rationale for the CVI in addition to the VS 1-27??? Tom

Thomas F.T. Linfield, DVM

Assistant District Director

District 5 Field Office for MT

USDA-APHIS-Veterinary Services

[208 N. Montana Ave](#); Suite 101

[Helena, MT 59601](#)

[\(406\) 449-2220](#)

[\(b\) \(6\)](#) (cell)

[\(406\) 449-5439](#) FAX

Thomas.F.Linfield@aphis.usda.gov

On Jun 16, 2015, at 4:34 PM, Frey, Rebecca K - APHIS

<Rebecca.K.Frey@aphis.usda.gov> wrote:

Thanks Tom,

However, Jack/ Pauline have been handling this with Dr. Roehr for several years and he always asks for a CVI. They have already informed him of the import.

Sent from my iPhone

On Jun 16, 2015, at 3:55 PM, Linfield, Thomas F - APHIS

<Thomas.F.Linfield@aphis.usda.gov> wrote:

Glen, Becky, Ryan, Brent:

Regarding sending bison from Corwin Springs to Ft. Collins, and the potential need for both CVI and VS Form 1-27: Glen stopped by the office today – we contacted Dr. Carl Heckendorf with the Colorado Department of Agriculture. Since the bison movement will be on a VS 1-27, he indicated a CVI was not necessary. He did however, request obtaining a Colorado Import number, and forwarding a scan of the VS 1-27.

Colorado Permit number: **303-869-9131**

He suggested sending a scan of the VS 1-27 to:

1) Keith Roehr: Keith.Roehr@state.co.us

2) Carl Heckendorf:

carl.heckendorf@state.co.us

3) Sunny Geiser-Novotny: [Sunny.Geiser-](mailto:Sunny.Geiser-Novotny@aphis.usda.gov)

Novotny@aphis.usda.gov

Thomas F.T. Linfield, DVM

Assistant District Director

District 5 Field Office for MT
USDA-APHIS-Veterinary Services
208 N. Montana Ave; Suite 101
Helena, MT 59601
(406) 449-2220
(b) (6) (cell)
(406) 449-5439 FAX
Thomas.F.Linfield@aphis.usda.gov

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: Re: Bison to CO - only VS 1-27 required by CO
Date: Wednesday, June 17, 2015 9:13:43 AM

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Sent from my iPhone

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Sent from my iPhone

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From: "Linfield, Thomas F - APHIS"
<Thomas.F.Linfield@aphis.usda.gov>
Date: June 16, 2015 at 9:20:23 PM MDT
To: "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>
Cc: "Bailey, Glen R - APHIS" <Glen.R.Bailey@aphis.usda.gov>, "Thompson, Brent D - APHIS" <Brent.D.Thompson@aphis.usda.gov>, "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>
Subject: Re: Bison to CO - only VS 1-27 required by CO

The question arose today, primarily because Glen is not deputized in m MT / does not have any MT DOL issued CVI's. We tried calling Dr Roehr to confirm - he was out of town, however Dr Heckendorf acting in his absence authorized VS 1-27 only, along with obtaining a CO permit. He actually had concerns regarding issuing a CVI with the "statements of health/ lack of exposure to infectious, contagious diseases on bison known to be infected or exposed to Brucella. ...

Did Dr Roehr provide any logical rationale for the CVI in addition to the VS 1-27??? Tom

Thomas F.T. Linfield, DVM

Assistant District Director

District 5 Field Office for MT

USDA-APHIS-Veterinary Services

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(406) 449-2220

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Thomas.F.Linfield@aphis.usda.gov

On Jun 16, 2015, at 4:34 PM, Frey, Rebecca K - APHIS
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Thanks Tom,
However, Jack/ Pauline have been handling this with Dr.
Roehr for several years and he always asks for a CVI.
They have already informed him of the import.

Sent from my iPhone

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He suggested sending a scan of the VS 1-27 to:

<!--[if !supportLists]-->1) <!--[endif]-->Keith

Roehr: Keith.Roehr@state.co.us

<!--[if !supportLists]-->2) <!--[endif]-->Carl

Heckendorf:

carl.heckendorf@state.co.us

<!--[if !supportLists]-->3) <!--[endif]-->

>Sunny Geiser-Novotny: Sunny.Geiser-Novotny@aphis.usda.gov

Thomas F.T. Linfield, DVM

Assistant District Director

District 5 Field Office for MT

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208 N. Montana Ave; Suite 101

Helena, MT 59601

(406) 449-2220

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(406) 449-5439 FAX

Thomas.F.Linfield@aphis.usda.gov

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Bison to NWRC
Date: Tuesday, June 23, 2015 11:49:00 AM

Yes. But you may have a handful of critters, born in 2013 probably orange tags with numbering something like 3R13 that would be sired by the SD bulls. You would have received them in January or shortly thereafter....in 2014 as coming yearlings.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Tuesday, June 23, 2015 11:46 AM
To: Frey, Rebecca K - APHIS
Cc: McCollum, Matthew P - APHIS
Subject: RE: Bison to NWRC

Oh yes. So the Red tagged bulls 59-69 are not products of the study but straight from the Park.

-----Original Message-----

From: Frey, Rebecca K - APHIS
Sent: Tuesday, June 23, 2015 11:41 AM
To: Nol, Pauline - APHIS
Subject: RE: Bison to NWRC

So, all negative bulls that we had extra last year, except for Red 59 who was a reactor. We received them in Feb of 2014 from YNP.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Monday, June 22, 2015 9:10 AM
To: Frey, Rebecca K - APHIS
Subject: FW: Bison to NWRC

Hey Becky,

Could you tell us again what the history is on the animals that we brought down on August 22, 2014?

Also, are there calves that we've inherited over the past few years that have come out of South Dakota bulls? Those would be animals that we'd be less likely to keep around, unless there was something scientifically interesting about them.

Thanks! Hope your weekend was good!

Pauline

-----Original Message-----

From: McCollum, Matthew P - APHIS
Sent: Friday, June 19, 2015 12:41 PM

To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Subject: FW: Bison to NWRC

Found it!

-----Original Message-----

From: McCollum, Matthew P - APHIS
Sent: Monday, August 25, 2014 2:27 PM
To: Clarke, Patrick R. - APHIS
Cc: Rhyan, Jack C - APHIS; keith.roehr@state.co.us; Linfield, Thomas F - APHIS; mzaluski@mt.gov; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: Re: Bison to NWRC

Bison received at the VS pens in Fort Collins. See attached.

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Bison to NWRC
Date: Tuesday, June 23, 2015 11:41:02 AM

So, all negative bulls that we had extra last year, except for Red 59 who was a reactor.
We received them in Feb of 2014 from YNP.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Monday, June 22, 2015 9:10 AM
To: Frey, Rebecca K - APHIS
Subject: FW: Bison to NWRC

Hey Becky,

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Sent: Friday, June 19, 2015 12:41 PM
To: Nol, Pauline - APHIS; Rhyen, Jack C - APHIS
Subject: FW: Bison to NWRC

Found it!

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Sent: Monday, August 25, 2014 2:27 PM
To: Clarke, Patrick R. - APHIS
Cc: Rhyen, Jack C - APHIS; keith.roehr@state.co.us; Linfield, Thomas F - APHIS; mzaluski@mt.gov; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: Re: Bison to NWRC

Bison received at the VS pens in Fort Collins. See attached.

From: [Clarke, Patrick R. - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); keith.roehr@state.co.us; [Linfield, Thomas F - APHIS](#); mzaluski@mt.gov; [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Bison to NWRC
Date: Friday, August 22, 2014 10:57:57 AM
Attachments: [1-27 Bison To NWRC 21 Aug 14.pdf](#)

1-27 accompanying this load.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, August 20, 2014 3:47 PM
To: 'Rhyan, Jack C - APHIS (Jack.C.Rhyan@aphis.usda.gov)'; keith.roehr; Linfield, Thomas F (APHIS); 'Zaluski, Martin' "Zaluski, Martin" Martin Zaluski (MZaluski@mt.gov); Frey, Rebecca K - APHIS; 'Pauline Nol'; McCollum, Matthew P - APHIS
Subject: Bison to NWRC

Please find attached a copy of a CVI (w/ permit number)for bison being transported from the GonaCon facility to NWRC (Ft Collins) on Friday August the 22nd.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES

FORM APPROVED
OMB NO. 0579-0051

No. F101899

PERMIT FOR MOVEMENT OF RESTRICTED ANIMALS

USE A SEPARATE FORM FOR EACH SPECIES

1. NAME AND ADDRESS OF SHIPPER OR CONSIGNOR (Include Zip Code)

APHIS Bison Quarantine Facility - Corral
772 Hwy 89S
Corwin Springs MT

2. CONSIGNEE (Destination Name and Address, include Zip Code)

USDA APHIS VS Research Pens
4101 La Porte Ave.
Fort Collins CO

3. MOVED FROM (Name and Location of Premise if other than item 1 above)

4. NAME AND ADDRESS OF OWNER AT TIME CONDITION DIAGNOSED

AS #1

5. STATE WHERE ISSUED

MT

6. MOVEMENT TO BE

☒ INTERSTATE ☐ INTRASTATE

7. MOVEMENT FOR

☒ QUARANTINE ☐ SLAUGHTER

8. DISEASE

Bruce's

9. STATUS OF ANIMALS

No. Reactor No. Exposed No. Other (Specify)

1

6

10. STATUS OF HERD OF ORIGIN

Infected

11. STATUS OF AREA OF ORIGIN

DSA

12. NO. ANIMALS IN THIS SHIPMENT

7

13. SPECIES (One only)

Bison

14. TRANSPORTATION VEHICLE LICENSE NO. OR OTHER IDENTIFICATION NO.

A312957

15. SEAL NO.

3785250

3785251

16. VEHICLE REQUIRED TO BE CLEANED AND DISINFECTED AT DESTINATION

☐ YES

☒ NO

(If Yes, Items 32, 33, and 34 are Applicable)

VALID ONLY FOR ABOVE DESTINATION

17. ANIMALS TO BE MOVED

COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)	COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)
81ASW3757	Bis	M	N/A	Red 69					
81ASW3760				Red 65					
81ASW3774				Red 61					
YNP930781				Red 63					
YNP930786				Red 66					
YNP930791				Red 59					
YNP930798				Red 62					

I certify that I have inspected the animals described on this permit and find them eligible to move in accordance with the requirements of State and Federal regulations.

18. SIGNATURE OF INSPECTOR

(b) (6)

19. DATE ISSUED

Aug 22, 14

20. TIME ISSUED

7:00AM

VOID AFTER

21. DATE

Aug 23, 2014

22. TIME

7:00AM

WARNING TO OWNER, SHIPPER AND TRUCKER - LIVESTOCK MUST BE DELIVERED TO CONSIGNEE WITHOUT DIVERSION

I understand that it is a violation of Federal law to move the animals identified herein interstate except in accordance with the provisions of applicable Federal Regulations. I also understand that such animals must comply with existing state laws and regulations governing movement of livestock and poultry. I have arranged or will arrange for a copy of this permit to accompany the interstate shipment and be delivered with the above described animals.

(b) (6)

24. TITLE

☒ OWNER

☐ SHIPPER

25. DATE SIGNED

22 Aug 2014

I certify that the animals described on this permit were received and slaughtered/quarantined in accordance with the requirements of the State and Federal regulations on the date indicated in item 29.

26. PLACE ANIMALS RECEIVED

27. DATE ANIMALS ARRIVED

28. NO. ANIMALS RECEIVED

29. DATE SLAUGHTERED/QUARANTINED

30. DATE AND TIME SEALS BROKE

31. AUTHORIZED SIGNATURE

32. DATE CLEANED AND DISINFECTED (if required)

33. SIGNATURE OF INSPECTOR

34. DATE SIGNED

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Bison to NWRC
Date: Tuesday, June 23, 2015 12:03:35 PM

I think the only animals of interest from that bunch were 3R21 and 3R13 since they were live births to dams where we actually cultured brucella in post birth samples.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Tuesday, June 23, 2015 11:53 AM
To: Frey, Rebecca K - APHIS
Cc: McCollum, Matthew P - APHIS
Subject: RE: Bison to NWRC

Okay, so the SD offspring were all born in 2013. We brought them in on Jan 9, 2014.
Thanks Becky!
Pauline

-----Original Message-----

From: Frey, Rebecca K - APHIS
Sent: Tuesday, June 23, 2015 11:49 AM
To: Nol, Pauline - APHIS
Subject: RE: Bison to NWRC

Yes. But you may have a handful of critters, born in 2013 probably orange tags with numbering something like 3R13 that would be sired by the SD bulls. You would have received them in January or shortly thereafter....in 2014 as coming yearlings.

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

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Cc: McCollum, Matthew P - APHIS
Subject: RE: Bison to NWRC

Oh yes. So the Red tagged bulls 59-69 are not products of the study but straight from the Park.

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To: Nol, Pauline - APHIS
Subject: RE: Bison to NWRC

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Veterinary Services
Montana

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Pauline

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From: McCollum, Matthew P - APHIS
Sent: Friday, June 19, 2015 12:41 PM
To: Nol, Pauline - APHIS; Rhyon, Jack C - APHIS
Subject: FW: Bison to NWRC

Found it!

-----Original Message-----

From: McCollum, Matthew P - APHIS
Sent: Monday, August 25, 2014 2:27 PM
To: Clarke, Patrick R. - APHIS
Cc: Rhyon, Jack C - APHIS; keith.roehr@state.co.us; Linfield, Thomas F - APHIS; mزالuski@mt.gov; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
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To: [Clarke, Patrick R. - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); keith.roehr@state.co.us; [Linfield, Thomas F - APHIS](#); mzaluski@mt.gov; [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: Bison to NWRC
Date: Monday, August 25, 2014 2:26:34 PM
Attachments: [ATT00001.txt](#)

Bison received at the VS pens in Fort Collins. See attached.

Thanks all,
Matt

Sent from my iPhone

> On Aug 22, 2014, at 10:57 AM, "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov> wrote:

>

> 1-27 accompanying this load.

>

> P. Ryan Clarke, DVM, MPH

> Regional Epidemiologist-GYA

> USDA, APHIS, VS, District 5

> 406-388-5162

>

> From: Clarke, Patrick R. - APHIS

> Sent: Wednesday, August 20, 2014 3:47 PM

> To: 'Rhyan, Jack C - APHIS (Jack.C.Rhyan@aphis.usda.gov)'; keith.roehr; Linfield, Thomas F (APHIS); "'Zaluski, Martin" <MZaluski@mt.gov> "Zaluski, Martin" Martin Zaluski (MZaluski@mt.gov)'; Frey, Rebecca K - APHIS; 'Pauline Nol'; McCollum, Matthew P - APHIS

> Subject: Bison to NWRC

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> P. Ryan Clarke, DVM, MPH

> Regional Epidemiologist-GYA

> USDA, APHIS, VS, District 5

> 406-388-5162

>

> <1-27 Bison To NWRC 21 Aug 14.pdf>

From: [Roehr - CDA, Keith](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: Re: Bison transport Montana to Fort Collins
Date: Friday, January 03, 2014 8:45:29 AM

Thanks Pauline, we'll issue a permit when we receive the VS 1-27. When you are ready to send the documents, call my office 303-239-4166 and if you don't reach me there try my cell (b) (6).

Keith A. Roehr DVM
Colorado State Veterinarian
303-239-4166

Please note that my email address has changed to : keith.roehr@state.co.us

On Thu, Jan 2, 2014 at 3:37 PM, Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov> wrote:

Dr. Roehr,

As per our conversation earlier today, we will be transporting between 10 and 20 bison from Montana to our Fort Collins Wildlife Research Facility between Wednesday and Friday of next week (January 8-10). These bison will be moved from our facility in Gardiner, MT, of which some may originate from trapping operations conducted this year. They will comprise of both Brucella positive and Brucella negative animals. These animals will be used for reproductive research in conjunction with CSU. Before transport we will send your office the VS-127 form, and if we are able, we also intend to send a Certificate of Veterinary Inspection.

Thanks you very much.

Pauline Nol

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: [970-266-6126](tel:970-266-6126)

Cell: (b) (6)

Fax: [970-266-6157](tel:970-266-6157)

This electronic message contains information generated by the USDA solely for the intended recipients. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the violator to civil or criminal penalties. If you believe you have received this message in error, please notify the sender and delete the email immediately.

Hi Keith,

Becky Frey was going to fax you the 1-27 but her copies did not pick up the carbon very well. I took a picture of the top copy and it is attached. Let me know if you cannot read it all and I will try again.

On Jan 3, 2014, at 8:45 AM, "Roehr - CDA, Keith" <keith.roehr@state.co.us> wrote:

Please note that my email address has changed to : keith.roehr@state.co.us

Dr Roehr,

Pauline Nol

003316

Fort Collins, CO 80521

Office: [970-266-6126](tel:970-266-6126)

Cell: (b) (6)

Fax: [970-266-6157](tel:970-266-6157)

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From: McCollum, Matthew P - APHIS
To: Rhyhan, Jack C - APHIS; Nol, Pauline - APHIS
Cc: Frey, Rebecca K - APHIS; Quance, Christine R - APHIS; Robbe Austerman, Suelee - APHIS; Clarke, Patrick R - APHIS
Subject: RE: BOFS semen and trans study
Date: Tuesday, April 09, 2013 3:50:09 PM
Attachments: image001.png

711 017 and red 03 would have all come out of the park in spring 2011. They may have been held in a capture pen together and had a common source of exposure. We still have 711. We euthanized 017 and will be sending the tissues from him in addition to blood and semen from 711. We plan to euthanize 711 later this spring and will collect tissues.

Matt and Jack

From: Rhyhan, Jack C - APHIS
Sent: Tuesday, April 09, 2013 3:29 PM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: BOFS semen and trans study
This is interesting!

j

From: Quance, Christine R - APHIS
Sent: Tuesday, April 09, 2013 11:39 AM
To: Frey, Rebecca K - APHIS; Rhyhan, Jack C - APHIS
Cc: Robbe Austerman, Suelee - APHIS; Clarke, Patrick R - APHIS
Subject: RE: BOFS semen and trans study
Hi Becky

Thanks for the reply. The reason we were asking is that we are looking at validation data for our WGS genotyping. We were specifically looking at isolates cultured repetitively from the same animal (for SNP call consistency) and noticed that the isolate from Red03 is an exact match to an isolate from animal 711 in 2011 and two isolates from animal 017 in 2012. This would be suggestive that all of these animals acquired *Brucella* from the same source or from each other. Perhaps they came out of the same area of the park...

reference_pos	73215	35778	777284	777285	413380	413381	954100	954101	102436	102437	102438	111796	111797	1352112	1352113	1352114	1352115	1352116	1352117	1352118	1352119	1352120	1352121	1352122	1352123	1352124	1352125	1352126	1352127	1352128	1352129	1352130	1352131	1352132	1352133	1352134	1352135	1352136	1352137	1352138	1352139	1352140	1352141	1352142	1352143	1352144	1352145	1352146	1352147	1352148	1352149	1352150	1352151	1352152	1352153	1352154	1352155	1352156	1352157	1352158	1352159	1352160	1352161	1352162	1352163	1352164	1352165	1352166	1352167	1352168	1352169	1352170	1352171	1352172	1352173	1352174	1352175	1352176	1352177	1352178	1352179	1352180	1352181	1352182	1352183	1352184	1352185	1352186	1352187	1352188	1352189	1352190	1352191	1352192	1352193	1352194	1352195	1352196	1352197	1352198	1352199	1352200	1352201	1352202	1352203	1352204	1352205	1352206	1352207	1352208	1352209	1352210	1352211	1352212	1352213	1352214	1352215	1352216	1352217	1352218	1352219	1352220	1352221	1352222	1352223	1352224	1352225	1352226	1352227	1352228	1352229	1352230	1352231	1352232	1352233	1352234	1352235	1352236	1352237	1352238	1352239	1352240	1352241	1352242	1352243	1352244	1352245	1352246	1352247	1352248	1352249	1352250	1352251	1352252	1352253	1352254	1352255	1352256	1352257	1352258	1352259	1352260	1352261	1352262	1352263	1352264	1352265	1352266	1352267	1352268	1352269	1352270	1352271	1352272	1352273	1352274	1352275	1352276	1352277	1352278	1352279	1352280	1352281	1352282	1352283	1352284	1352285	1352286	1352287	1352288	1352289	1352290	1352291	1352292	1352293	1352294	1352295	1352296	1352297	1352298	1352299	1352300	1352301	1352302	1352303	1352304	1352305	1352306	1352307	1352308	1352309	1352310	1352311	1352312	1352313	1352314	1352315	1352316	1352317	1352318	1352319	1352320	1352321	1352322	1352323	1352324	1352325	1352326	1352327	1352328	1352329	1352330	1352331	1352332	1352333	1352334	1352335	1352336	1352337	1352338	1352339	1352340	1352341	1352342	1352343	1352344	1352345	1352346	1352347	1352348	1352349	1352350	1352351	1352352	1352353	1352354	1352355	1352356	1352357	1352358	1352359	1352360	1352361	1352362	1352363	1352364	1352365	1352366	1352367	1352368	1352369	1352370	1352371	1352372	1352373	1352374	1352375	1352376	1352377	1352378	1352379	1352380	1352381	1352382	1352383	1352384	1352385	1352386	1352387	1352388	1352389	1352390	1352391	1352392	1352393	1352394	1352395	1352396	1352397	1352398	1352399	1352400	1352401	1352402	1352403	1352404	1352405	1352406	1352407	1352408	1352409	1352410	1352411	1352412	1352413	1352414	1352415	1352416	1352417	1352418	1352419	1352420	1352421	1352422	1352423	1352424	1352425	1352426	1352427	1352428	1352429	1352430	1352431	1352432	1352433	1352434	1352435	1352436	1352437	1352438	1352439	1352440	1352441	1352442	1352443	1352444	1352445	1352446	1352447	1352448	1352449	1352450	1352451	1352452	1352453	1352454	1352455	1352456	1352457	1352458	1352459	1352460	1352461	1352462	1352463	1352464	1352465	1352466	1352467	1352468	1352469	1352470	1352471	1352472	1352473	1352474	1352475	1352476	1352477	1352478	1352479	1352480	1352481	1352482	1352483	1352484	1352485	1352486	1352487	1352488	1352489	1352490	1352491	1352492	1352493	1352494	1352495	1352496	1352497	1352498	1352499	1352500	1352501	1352502	1352503	1352504	1352505	1352506	1352507	1352508	1352509	1352510	1352511	1352512	1352513	1352514	1352515	1352516	1352517	1352518	1352519	1352520	1352521	1352522	1352523	1352524	1352525	1352526	1352527	1352528	1352529	1352530	1352531	1352532	1352533	1352534	1352535	1352536	1352537	1352538	1352539	1352540	1352541	1352542	1352543	1352544	1352545	1352546	1352547	1352548	1352549	1352550	1352551	1352552	1352553	1352554	1352555	1352556	1352557	1352558	1352559	1352560	1352561	1352562	1352563	1352564	1352565	1352566	1352567	1352568	1352569	1352570	1352571	1352572	1352573	1352574	1352575	1352576	1352577	1352578	1352579	1352580	1352581	1352582	1352583	1352584	1352585	1352586	1352587	1352588	1352589	1352590	1352591	1352592	1352593	1352594	1352595	1352596	1352597	1352598	1352599	1352600	1352601	1352602	1352603	1352604	1352605	1352606	1352607	1352608	1352609	1352610	1352611	1352612	1352613	1352614	1352615	1352616	1352617	1352618	1352619	1352620	1352621	1352622	1352623	1352624	1352625	1352626	1352627	1352628	1352629	1352630	1352631	1352632	1352633	1352634	1352635	1352636	1352637	1352638	1352639	1352640	1352641	1352642	1352643	1352644	1352645	1352646	1352647	1352648	1352649	1352650	1352651	1352652	1352653	1352654	1352655	1352656	1352657	1352658	1352659	1352660	1352661	1352662	1352663	1352664	1352665	1352666	1352667	1352668	1352669	1352670	1352671	1352672	1352673	1352674	1352675	1352676	1352677	1352678	1352679	1352680	1352681	1352682	1352683	1352684	1352685	1352686	1352687	1352688	1352689	1352690	1352691	1352692	1352693	1352694	1352695	1352696	1352697	1352698	1352699	1352700	1352701	1352702	1352703	1352704	1352705	1352706	1352707	1352708	1352709	1352710	1352711	1352712	1352713	1352714	1352715	1352716	1352717	1352718	1352719	1352720	1352721	1352722	1352723	1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B12-0460V_12BA1_CO-069_BI-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	C	T	T	T	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	A	T	T	T	A	G	C	C	C	C	G	A	A	C	T	G	C	T	T	G	C	A	C	G	C	C	G		017	
B12-0560_12BA1_CO-069_BI-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	C	T	T	T	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	A	T	T	T	A	G	C	C	C	C	G	A	A	C	T	G	C	T	T	G	C	A	C	G	C	C	G		017	
B13-0061V_13BA1_MT-067_BI-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	C	T	T	T	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	A	T	T	T	A	G	C	C	C	C	G	A	A	C	T	G	C	T	T	G	C	A	C	G	C	C	G		Red03	
B11-0469V_11BA1_MT-XXX_BI-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	T	T	T	A	G	A	A	C	T	G	C	T	T	G	C	A	C	G	C	C	G			
B10-0118V_09BA1_MT-067_EK-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	A	C	G	A	C		G	C	T	T	G	C	A	C	G	C	C	G		
B10-0273V_09BA1_MT-067_EK-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	A	T	G	C	G	C	C	G			
B08-0416V_08BA1_MT-067_CA-08A1	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	C	C	G			
B10-0529V_09BA1_MT-067_EK-WLx	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	A	C	C	G			
B11-0604V_11BA1_MT-067_CA-11A1	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	T	T	C	G		
B11-0612V_MP-IG_11BA1_MT-067_CA-11A3	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	T	T	C	G		
B11-0616V_11BA1_MT-067_CA-11A5	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	T	T	C	G		
B11-0611V_11BA1_MT-067_CA-11A2	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	T	T	A	G		
B11-0613V_11BA1_MT-067_CA-11A4	A	C	G	T	G	C	C	G	G	C	A	T	G	G	G	G	G	T	C	C	G	T	G	A	C	G	A	G	C	C	T	A	C	G	G	G	C	C	T	G	G	G	G	T	T	C	G	A	G	C	C	A	G	C	C	C	C	C	G	A	A	C	T	T	T	C	G	G	C	A	T	G	T	T	C	A		

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Brian J McCluskey](#); (b) (6); [Lowell A Miller/CO/APHIS/USDA](#); [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: brief protocol for bison immunocontraceptive project
Date: Tuesday, June 22, 2010 9:28:00 AM

Just a thought ...we'll need to get ~23 seronegative heifers from the trap to compensate for the one that will seroconvert after they get to the BQFS facility.

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell
□ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

06/16/2010 12:36 PM

To Brian J
McCluskey/CO/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA,
Lowell A Miller/CO/APHIS/USDA@USDA,
(b) (6) @gmail.com>
cc Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA
Subject: brief protocol for bison immunocontraceptive
project

All,
Attached is a brief protocol I'd like to send to Jack Edmundson for them to start work on. It will undoubtedly require an EA with public meetings. This will be enough for them to start with. Please review and correct, expand, etc.
Thanks much.
Jack
[attachment "ImmunocontBisonProject.doc" deleted by Patrick R Clarke/MT/APHIS/USDA]

From: [Pauline Nol](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Patrick R Clarke](#); [Rebecca K Frey](#)
Subject: Re: brief protocol of immunocontraceptive study
Date: Wednesday, October 13, 2010 3:58:00 PM

Looks good to me too.
Pauline

☐ Jack C Rhyan---10/13/2010 03:23:09 PM---Please make any changes and I'll incorporate them. I'll then send it on to Brian and, with his approval, on to Jack E. Thanks,

**Jack C
Rhyan/CO/APHIS/USDA**

10/13/2010 03:26 PM

ToPatrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subjectbrief protocol of immunocontraceptive study

Please make any changes and I'll incorporate them. I'll then send it on to Brian and, with his approval, on to Jack E.

Thanks,

Jack[attachment "ImmunocontBisonProject_10-13.doc" deleted by Pauline
Nol/CO/APHIS/USDA]

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: brief protocol of immunocontraceptive study
Date: Wednesday, October 13, 2010 3:48:00 PM

Looks good.

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell
□ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

10/13/2010 03:26 PM

ToPatrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subjectbrief protocol of immunocontraceptive study

Please make any changes and I'll incorporate them. I'll then send it on to Brian and, with his approval, on to Jack E.

Thanks,

Jack[attachment "ImmunocontBisonProject_10-13.doc" deleted by Patrick R
Clarke/MT/APHIS/USDA]

From: [Rebecca K Frey](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Patrick R Clarke](#); [Pauline Nol](#)
Subject: Re: brief protocol of immunocontraceptive study
Date: Thursday, October 14, 2010 9:40:00 AM

Who is Luke Wagner?

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425
(b) (6) cell

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: RE: CC about excess calves
Date: Monday, November 24, 2014 12:07:00 PM

8 and 10 work for me as well as the afternoon of the 9th.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Monday, November 24, 2014 11:13 AM
To: Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Subject: CC about excess calves

Hi everybody!

Ryan and I were chatting and decided we should have a conference call to discuss the excess calves from GonaCon, who has space, what needs we have for following offspring etc. Seems like we have done this, but I feel like with the space issues, we are letting some things go that are not the priority of the study. So can we figure out if there are true needs of the study that require us to keep some of the offspring, and then which ones are most important? We plan on keeping a couple of bulls each year, just to make sure we always have bulls, so we can get rid of the big nasty ones as they age.

Anytime in December.....except 10 am on the 5th, or the morning of 16th for me.

Can we shoot for a date 8th 9th or 10th to begin the negotiating?

Thanks,

Becky

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#); [Rhyen, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Re: Cheat sheet and data sheet for VOC stuff
Date: Wednesday, February 19, 2014 12:15:19 PM

Loads of young stuff! We are getting 6 2yo cows, and some bulls, not done testing yet.... For Gonacon

Becky
USDA APHIS VS
Sent from my iPhone

On Feb 19, 2014, at 9:52 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Thanks for the update Becky!
54 animals would have been great, but such is life...
When you have time, let's do a video call if you can this week. I just found out that we can do that, kind of like skype but it's called cisco webex. That may be the best way to go through all the VOC stuff. I just need to furnish time and duration and then our security guy, Tony Perretta, can set it up for us. Ideally you guys would just have to click on a link and voila!
Glad the VIT's aren't on the ground again, wherever they are!
Pauline

From: Frey, Rebecca K - APHIS
Sent: Wednesday, February 19, 2014 8:58 AM
To: Nol, Pauline - APHIS
Cc: Clarke, Patrick R. - APHIS; Rhyen, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: Re: Cheat sheet and data sheet for VOC stuff
Hi. Ryan and I should be able to cover the earlier testing when it occurs, as long as we can figure out all of the instructions! And I think we will have Brent so hopefully we can cover testing and any slaughter sampling for a day or two. We are testing 54 hd today. Hoping for some non preg girls, but I am no pro preg checker!!!
Talk to ya later with an update..... My VITs lasted all night! I must of gotten them close to right, or at least crammed in the bladder. :-)

Becky
USDA APHIS VS
Sent from my iPhone

On Feb 18, 2014, at 3:41 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Hey there,
I forgot to print out the data sheet and cheat sheet to include in the shipment of buckets which should arrive on your doorstep tomorrow, Becky. Just in case you guys are able to take the samples for us, I'm

sending these to you. I will try to make it up there next week but it probably won't be until Wednesday if there is a plan to test on Thursday. Maybe we can go over it on the phone???

Thanks!

Pauline

From: [O'Hare, Jeanette R \(APHIS\)](#)
To: [Eisemann, John D \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#)
Cc: [Stephens, Stephanie H \(APHIS\)](#)
Subject: RE: comments on bison protocol
Date: Monday, June 06, 2011 1:43:53 PM

Just a note to concur with John's comment in the protocol regarding the GonaCon formulation. What you have in the protocol right now is the currently registered product. Lowell has made several changes for a new formulation which have significant regulatory implications. We need to clarify this.

Jeanette

From: Eisemann, John D (APHIS)
Sent: Monday, June 06, 2011 11:03 AM
To: Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS); O'Hare, Jeanette R (APHIS)
Subject: comments on bison protocol

I am around all week if you want to discuss any of these comments.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: [Eisemann, John D \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [O'Hare, Jeanette R \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#)
Cc: [Stephens, Stephanie H \(APHIS\)](#)
Subject: RE: comments on bison protocol
Date: Monday, June 06, 2011 2:05:54 PM

Please talk to Lowell about this. Include either me or Jeanette in the discussion.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: Nol, Pauline (APHIS)
Sent: Monday, June 06, 2011 1:45 PM
To: O'Hare, Jeanette R (APHIS); Eisemann, John D (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

I hijacked that information from the elk protocol. This can be changed however it needs to be changed.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: O'Hare, Jeanette R (APHIS)
Sent: Monday, June 06, 2011 1:44 PM
To: Eisemann, John D (APHIS); Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

Just a note to concur with John's comment in the protocol regarding the GonaCon formulation. What you have in the protocol right now is the currently registered product. Lowell has made several changes for a new formulation which have significant regulatory implications. We need to clarify this.

Jeanette

From: Eisemann, John D (APHIS)
Sent: Monday, June 06, 2011 11:03 AM
To: Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS); O'Hare, Jeanette R (APHIS)
Subject: comments on bison protocol

I am around all week if you want to discuss any of these comments.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Clarke, Patrick R. \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: RE: Conf call tomorrow
Date: Tuesday, July 19, 2011 12:09:41 PM

I'm in.

From: Clarke, Patrick R. (APHIS)
Sent: Tuesday, July 19, 2011 11:45 AM
To: Rhyan, Jack C (APHIS); Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS); Nol, Pauline (APHIS)
Cc: Clarke, Patrick R. (APHIS)
Subject: Conf call tomorrow

All:

Can we have a conf call tomorrow(July 20) at 10:15 am?

Subject: recent serologic results for GonaCon bison----three bulls seropositive

Call in #: (b) (6)

Code: (b) (6)

Cheers,
Ryan

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

From: [Frey, Rebecca K - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 3:41:45 PM

Chances are we have 8 hd in this category. Lets go straight to commercial option. FWP will run from this, they want no part of buffalo.

Becky
USDA APHIS VS
Sent from my iPhone

On Feb 12, 2013, at 3:23 PM, "Rhyan, Jack C - APHIS" <Jack.C.Rhyan@aphis.usda.gov> wrote:

[Forgot to cc ya'll. Sorry.](#)

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 3:21 PM
To: Clarke, Patrick R. - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

Off the top of my bald head:

I like the bastard question best. I think with those we donate their little bastard carcasses to the food bank, as they have no special value for conservation.
As for the dumb question: The FWP thing didn't work out so well last time. We carefully avoided the issue of ownership by calling them "wildlife" which was our desire. However, reality has conquered desire and now I just want to avoid the political games if we can. We found several homes for them last time only to be shot down by the FWP thing. I think this time if we can find a state park or tribe well ahead of time, we'll transfer the critters to them. Maybe we'll ask to get bloods once in the next 5 years as a follow-up surveillance. If things get sticky, we'll utilize the last option and collect tissues from them looking for latent infection.

What do you think about those random thoughts?

Jack

PS: we might should delete these emails.

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 2:52 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

This may be a dumb question..... but why aren't the seronegative bison that graduate from the GonaCon study (and have met the BQFS protocol)....why aren't they wildlife and the property of FWP just like the BQFS graduates? What makes them different when they came from the same source?

Which brings up another question....some females were bred by SD bulls.....what do we do with their negative offspring?(i.e. the impure Yellowstone bastards!)

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 12:14 PM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
2 pm today will work for a talk with Rick and PJ. I will call you all from here. Okay?
Jack

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 10:06 AM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
I'm home. We are talking about two conference calls now. I haven't heard back from
Matt about times for our inter-APHIS on the 19th or 20th.
As for a CC with YNP.....I'm available for the rest of today and after 3pm tomorrow.
Ryan
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 9:25 AM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
What times today or tomorrow are you available? Is Ryan still at Plum?
Jack

From: Frey, Rebecca K - APHIS
Sent: Tuesday, February 12, 2013 9:21 AM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Probably not until Thursday, so if there is a pressing time issue we should do a CC
Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 9:20 AM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

So maybe the best thing is if we get on the phone with Rick and PJ and hash out our concerns. I'm sure they are trying to make it acceptable to whoever in NPS is grouchy about giving animals away, but I think you two both have good points. Can you guys get to Mammoth for a face to face with me on the phone? Or should we try to organize a conf call?

Jack

From: Frey, Rebecca K - APHIS

Sent: Tuesday, February 12, 2013 8:57 AM

To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS

Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

I think once they give us "ownership" they no longer get to dictate the terms of the disposition. We already stated in our plan that we will attempt to place appropriate bison, but we should not be told by them what to do. I agree that we need dates....or x number of days/months after the study ends to dispose of them.

Also, as to use of any bulls, they do state the pastures at "Corwin Springs", so if they can only be there....we could not even move them to CO, unless we feel like the movement to Corwin only means "initial" movement from Park. Probably need to edit Article VI part D by removing "this" in front of research....so it doesn't limit us to the GonaCon Study.

Did we pick a time yet for the conference call?

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: Clarke, Patrick R. - APHIS

Sent: Tuesday, February 12, 2013 8:28 AM

To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS

Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

They give us ownership in article VI (A), but then dictate what happens to the bison 4-5 years later. Remember how we were stuck feeding and caring for BQFS for a year+, because we could not get rid of them. A think there should be another clause that says something like

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We do not want to be stuck with thse animal for months and months and months with out certain deadlines in place.

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA-APHIS-VS-WR

406-388-5162

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Sent: Monday, February 11, 2013 4:15 PM

To: Frey, Rebecca K - APHIS

Cc: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

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Thanks for the scrutiny, Becky, Esquire.

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Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

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Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

Before noon on 20th, or before 2:30 on 19th

Becky
USDA APHIS VS
Sent from my iPhone

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<Patrick.R.Clarke@aphis.usda.gov> wrote:

All,
I think we need to put our heads together about what we want to do
about the Brogan facility, the 2nd rendition of GonaCon, an elk study, etc.
What is everyone availability next Tuesday (19th) or Wednesday (20th) to
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P. Ryan Clarke, DVM, MPH
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406-388-5162

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Wednesday, February 13, 2013 3:50:00 PM

I actually goofed and won't be available until 1pm. So if it's a pain to change the time, y'all can just fill me in later.

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, February 13, 2013 3:44 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Whoops....Pauline is not available until 11 am.....so it's 11 am on the 19th. Do we need to let Deb Beaugh that the time has been changed?
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 1:31 PM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
The 19th at 9.

From: Beaugh, Debra A - APHIS
Sent: Tuesday, February 12, 2013 1:17 PM
To: Rhyan, Jack C - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
You're all set for it. The phone number is (b) (6). The Host PW is: (b) (6). The access code is: (b) (6)
Let me know if you need anything else.
Deb

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 12:24 PM
To: Beaugh, Debra A - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Feb 19th at 9 am for about an hour or less.
Jack

From: Beaugh, Debra A - APHIS
Sent: Tuesday, February 12, 2013 11:38 AM
To: Rhyan, Jack C - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Dr. Rhyan,
When do you want to use it?
Deb

From: Strang, Penny M - APHIS
Sent: Tuesday, February 12, 2013 11:27 AM
To: Rhyan, Jack C - APHIS; Beaugh, Debra A - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Hi Debbie.

Can you answer Dr. Rhyan's question re: Dr. Herriott's conference call number? Thanks!

*Penny Strang
Administrative Support Assistant (Procurement)
USDA APHIS Veterinary Services
2150 Centre Ave., Bldg. B
Fort Collins, CO 80526
Ph. 970-494-7386*

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 11:24 AM
To: Strang, Penny M - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Penny,
Can we use Don's conf call number for a call with Becky, Ryan and some YNP folks?
Jack

From: Frey, Rebecca K - APHIS
Sent: Tuesday, February 12, 2013 11:20 AM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
This is Don's number.....can we use it?
(b) (6); **Passcode** (b) (6) (leader passcode is: (b) (6))

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 11:07 AM
To: Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
We can do that.
I'm still trying to get Rick.
Jack

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 10:58 AM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
How about 9 am on the 19th. Do you guys have a conference phone we can call into? I will be in NV.
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: McCollum, Matthew P - APHIS
Sent: Tuesday, February 12, 2013 10:25 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS
Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
I'm available whenever. Just have to be at the hub to pick up my new badge at 11 tomorrow.

Sent from my handheld phone.

From: Clarke, Patrick R. - APHIS

Sent: Tuesday, February 12, 2013 05:06 PM

To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS

Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

I'm home. We are talking about two conference calls now. I haven't heard back from Matt about times for our inter-APHIS on the 19th or 20th.

As for a CC with YNP.....I'm available for the rest of today and after 3pm tomorrow.

Ryan

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA-APHIS-VS-WR

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What times today or tomorrow are you available? Is Ryan still at Plum?

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Probably not until Thursday, so if there is a pressing time issue we should do a CC

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Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 3:41:45 PM

Chances are we have 8 hd in this category. Lets go straight to commercial option. FWP will run from this, they want no part of buffalo.

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Off the top of my bald head:

I like the bastard question best. I think with those we donate their little bastard carcasses to the food bank, as they have no special value for conservation.
As for the dumb question: The FWP thing didn't work out so well last time. We carefully avoided the issue of ownership by calling them "wildlife" which was our desire.
However, reality has conquered desire and now I just want to avoid the political games if we can. We found several homes for them last time only to be shot down by the FWP thing. I think this time if we can find a state park or tribe well ahead of time, we'll transfer the critters to them. Maybe we'll ask to get bloods once in the next 5 years as a follow-up surveillance. If things get sticky, we'll utilize the last option and collect tissues from them looking for latent infection.

What do you think about those random thoughts?

Jack

PS: we might should delete these emails.

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Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
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This may be a dumb question..... but why aren't the seronegative bison that graduate from the GonaCon study (and have met the BQFS protocol)....why aren't they wildlife and the property of FWP just like the BQFS graduates? What makes them different when they came from the same source?

Which brings up another question....some females were bred by SD bulls.....what do we do with their negative offspring?(i.e. the impure Yellowstone bastards!)

P. Ryan Clarke, DVM, MPH

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Sent from my iPhone

On Feb 11, 2013, at 12:31 PM, "Clarke, Patrick R. - APHIS"
<Patrick.R.Clarke@aphis.usda.gov> wrote:

All,
I think we need to put our heads together about what we want to do
about the Brogan facility, the 2nd rendition of GonaCon, an elk study, etc.
What is everyone availability next Tuesday (19th) or Wednesday (20th) to
have a conference call?
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Wednesday, February 13, 2013 3:50:00 PM

I actually goofed and won't be available until 1pm. So if it's a pain to change the time, y'all can just fill me in later.

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, February 13, 2013 3:44 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Whoops....Pauline is not available until 11 am.....so it's 11 am on the 19th. Do we need to let Deb Beaugh that the time has been changed?
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 1:31 PM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
The 19th at 9.

From: Beaugh, Debra A - APHIS
Sent: Tuesday, February 12, 2013 1:17 PM
To: Rhyan, Jack C - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
You're all set for it. The phone number is: (b) (6). The Host PW is: (b) (6). The access code is: (b) (6).
Let me know if you need anything else.
Deb

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 12:24 PM
To: Beaugh, Debra A - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Feb 19th at 9 am for about an hour or less.
Jack

From: Beaugh, Debra A - APHIS
Sent: Tuesday, February 12, 2013 11:38 AM
To: Rhyan, Jack C - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Dr. Rhyan,
When do you want to use it?
Deb

From: Strang, Penny M - APHIS
Sent: Tuesday, February 12, 2013 11:27 AM
To: Rhyan, Jack C - APHIS; Beaugh, Debra A - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Hi Debbie.

Can you answer Dr. Rhyan's question re: Dr. Herriott's conference call number? Thanks!

*Penny Strang
Administrative Support Assistant (Procurement)
USDA APHIS Veterinary Services
2150 Centre Ave., Bldg. B
Fort Collins, CO 80526
Ph. 970-494-7386*

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 11:24 AM
To: Strang, Penny M - APHIS
Subject: FW: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Penny,

Can we use Don's conf call number for a call with Becky, Ryan and some YNP folks?

Jack

From: Frey, Rebecca K - APHIS
Sent: Tuesday, February 12, 2013 11:20 AM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
This is Don's number.....can we use it?

(b) (6); **Passcode** (b) (6) (leader passcode is: (b) (6))

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyan, Jack C - APHIS
Sent: Tuesday, February 12, 2013 11:07 AM
To: Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
We can do that.

I'm still trying to get Rick.

Jack

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 10:58 AM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
How about 9 am on the 19th. Do you guys have a conference phone we can call into? I will be in NV.
P. Ryan Clarke, DVM, MPH
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From: McCollum, Matthew P - APHIS
Sent: Tuesday, February 12, 2013 10:25 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS
Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
I'm available whenever. Just have to be at the hub to pick up my new badge at 11 tomorrow.

Sent from my handheld phone.

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 05:06 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
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Also, as to use of any bulls, they do state the pastures at "Corwin Springs", so if they can only be there..we could not even move them to CO, unless we feel like the movement to Corwin only means "initial" movement from Park. Probably need to edit Article VI part D by removing "this" in front of research..so it doesn't limit us to the GonaCon Study.

Did we pick a time yet for the conference call?

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

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406-333-4425

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Becky, will you be my lawyer? I think you could keep me out of trouble.

I guess I read the "and/or" and thought we could live with it but maybe I'll take it out. I think if I add "or paddock" to pasture, we could bring them here. What do you think?

Thanks for the scrutiny, Becky, Esquire.

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especially if you have ANY other research or samples you would want to take from them, ie.embryo transfer, semen collection etc...I think since you are considered "KEY".what a word.for both facilities, we could probably send them, it is just that they will no longer be part of the GonaCon study.

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Before noon on 20th, or before 2:30 on 19th

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Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 3:54:42 PM

Do we have a genetic test we are comfortable with? If so, we should collect samples for that testing as well. If not, then the commercial option would be the way to go.

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Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 1:14:58 PM

I can jump in when done.
Thanks Matt!

From: McCollum, Matthew P - APHIS
Sent: Tuesday, February 12, 2013 07:27 PM
To: Rhyan, Jack C - APHIS
Cc: Nol, Pauline - APHIS
Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

Pauline has that pig meeting this aft...
Sent from my handheld phone.

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Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
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Montana
406-333-4425

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Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

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Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 3:40:13 PM

As far as what makes them different... I guess it would just be the agreement that they were transferred under.

The bison from SD were reputed to be "pure" we can test the calves and use them for conservation if they conform to the standard.

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, February 12, 2013 2:52 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
This may be a dumb question..... but why aren't the seronegative bison that graduate from the GonaCon study (and have met the BQFS protocol)....why aren't they wildlife and the property of FWP just like the BQFS graduates? What makes them different when they came from the same source?
Which brings up another question.....some females were bred by SD bulls.....what do we do with their negative offspring?(i.e. the impure Yellowstone bastards!)
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Cc: [Nol, Pauline - APHIS](#)
Subject: Re: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 10:24:35 AM

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Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 1:35:09 PM

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Date: Tuesday, February 12, 2013 12:13:55 PM

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Date: Tuesday, February 12, 2013 11:23:19 AM

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Date: Tuesday, February 12, 2013 11:06:48 AM

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Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?
Date: Tuesday, February 12, 2013 10:06:28 AM

I'm home. We are talking about two conference calls now. I haven't heard back from Matt about times for our inter-APHIS on the 19th or 20th.

As for a CC with YNP.....I'm available for the rest of today and after 3pm tomorrow.

Ryan

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Date: Tuesday, February 12, 2013 9:20:03 AM

So maybe the best thing is if we get on the phone with Rick and PJ and hash out our concerns. I'm sure they are trying to make it acceptable to whoever in NPS is grouchy about giving animals away, but I think you two both have good points. Can you guys get to Mammoth for a face to face with me on the phone? Or should we try to organize a conf call?

Jack

From: Frey, Rebecca K - APHIS
Sent: Tuesday, February 12, 2013 8:57 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS
Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
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Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

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Before noon on 20th, or before 2:30 on 19th

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[P. Ryan Clarke, DVM, MPH](#)
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

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To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
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Date: Monday, February 11, 2013 1:51:10 PM

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Hi Debbie.

Can you answer Dr. Rhyan's question re: Dr. Herriott's conference call number? Thanks!

Penny Strang

Administrative Support Assistant (Procurement)

USDA APHIS Veterinary Services

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Montana
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Also, as to use of any bulls, they do state the pastures at "Corwin Springs", so if they can only be there..we could not even move them to CO, unless we feel like the movement to Corwin only means "initial" movement from Park. Probably need to edit Article VI part D by removing "this" in front of research..so it doesn't limit us to the GonaCon Study.

Did we pick a time yet for the conference call?

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They give us ownership in article VI (A), but then dictate what happens to the bison 4-5 years later. Remember how we were stuck feeding and caring for BQFS for a year+, because we could not get rid of them. A think there should be another clause that says something like
" APHIS will give YNP as much notice as possible as to when certain bison will be leaving the study. It will be YNP responsibility to find suitable parties [as dictated in Article VI (B) & (C)] to accept the

eligible bison by the date of their redundancy. If YNP cannot find suitable a suitable party by the redundancy date, APHIS will exercise it's default for excess research animals which is to have a local slaughterhouse process the animals and give the meat to the Montana Food Bank Network. We do not want to be stuck with thse animal for months and months and months with out certain deadlines in place.

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Becky, will you be my lawyer? I think you could keep me out of trouble.
I guess I read the "and/or" and thought we could live with it but maybe I'll take it out. I think if I add "or paddock" to pasture, we could bring them here. What do you think?
Thanks for the scrutiny, Becky, Esquire.
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You don't have any heartburn about the "statement of work" stating overectomy as a part of the study, the preferences on disposal of bison at end of study, or the limits on where the bison can be used for research? My first blush would keep us from sending sero-positive bulls to you in CO, especially if you have ANY other research or samples you would want to take from them, ie.embryo transfer, semen collection etc...I think since you are considered "KEY".what a word.for both facilities, we could probably send them, it is just that they will no longer be part of the GonaCon study.
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Subject: RE: conference call
Date: Monday, April 07, 2014 4:35:57 PM

That's the way I remember it.

Jack

From: Nol, Pauline - APHIS
Sent: Monday, April 07, 2014 3:58 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: conference call

Hi there,

We decided on vaccinating 20 of the positive animals, correct? I'm trying to get together a GonaCon order for May.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, March 25, 2014 1:01 PM
To: Nol, Pauline - APHIS
Subject: RE: conference call

We have 25 pos females, 22 of which are 2yo and 3yo's.

10 Neg F prior to last weeks test.

20 Neg Males prior to last weeks test.

Rebecca Frey

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Montana
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From: Nol, Pauline - APHIS
Sent: Monday, March 24, 2014 2:04 PM
To: Frey, Rebecca K - APHIS
Subject: RE: conference call

Hey Becky,

Could you send the final numbers for how many new bison we got for Gonacon? So I can pretend I'm prepared for tomorrow's meeting;)

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Oh I suppose. :-). 2 on Tuesday.... That's tomorrow.

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On Mar 24, 2014, at 9:59 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Can we move to 2pm?
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Sent: Friday, March 21, 2014 11:35 AM
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USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
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Regional Epidemiologist-GYA
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Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: conference call
Date: Tuesday, March 25, 2014 1:01:15 PM

We have 25 pos females, 22 of which are 2yo and 3yo's.

10 Neg F prior to last weeks test.

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Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

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To: Frey, Rebecca K - APHIS
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Becky

USDA APHIS VS

Sent from my iPhone

On Mar 24, 2014, at 9:59 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Can we move to 2pm?

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To: [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: conference call
Date: Tuesday, March 25, 2014 7:29:41 AM

Time 2 PM today.

Call: (b) (6)

Passcode: (b) (6)

Why? GonaCon! Well, actually, we just like talking about brucellosis.....

Hear ya then!

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: Nol, Pauline - APHIS
Sent: Monday, March 24, 2014 10:43 AM
To: Frey, Rebecca K - APHIS
Cc: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: conference call
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Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: conference call
Date: Monday, March 24, 2014 10:42:00 AM

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Subject: RE: conference call
Date: Monday, March 24, 2014 9:58:00 AM

Can we move to 2pm?

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Subject: RE: conference call
Date: Friday, March 21, 2014 11:34:00 AM

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To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: conference call
Date: Monday, April 07, 2014 9:00:49 PM

Yes, that sounds right to me.

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Monday, April 07, 2014 3:58 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: FW: conference call

Hi there,

We decided on vaccinating 20 of the positive animals, correct? I'm trying to get together a GonaCon order for May.

Thanks!

Pauline
Pauline Nol, DVM, MS, PhD
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20 Neg Males prior to last weeks test.

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Monday or Tuesday work for me at any of the times. Wednesday is out.
Thanks,
Jack

From: Nol, Pauline - APHIS
Sent: Friday, March 21, 2014 11:35 AM

To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: conference call

I'm pretty open those days too.

I think Molly just wants to talk with folks and get a feel for what ya'll do. If there is something to participate in then wonderful but otherwise no stress.

I do have a vet student coming for an externship in about two weeks who could theoretically come up for a week and help with potential calvings if possible. Maybe he could ride with Brent a bit too, he he.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS
Sent: Friday, March 21, 2014 9:27 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: conference call

Any of those dates and times good for me

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Frey, Rebecca K - APHIS
Sent: Friday, March 21, 2014 9:12 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: conference call

Can we schedule a call for next week, Tuesday or Wednesday, to discuss the GonaCon project moving forward? Pauline, maybe we can talk about the student request too and figure something out.

I will throw out 1, 2 or 3 pm either day as a starting point.

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Rhyan, Jack C. \(APHIS\)](#)
To: [Stephens, Stephanie H. \(APHIS\)](#)
Cc: [McCollum, Matthew P. \(APHIS\)](#); [Frey, Rebecca K. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#)
Subject: RE: Consumption of Bison from GonaCon Experiment
Date: Tuesday, July 26, 2011 11:24:13 AM

Stephanie,

I find this a little crazy since the vaccine is approved by EPA for use in wild deer. Are they ever consumed? Anyway, if that is what they want, we will assure them that the vaccinates (not the controls or bulls or calves) will not be consumed.

Jack

From: Stephens, Stephanie H (APHIS)
Sent: Tuesday, July 26, 2011 9:05 AM
To: Rhyan, Jack C (APHIS)
Subject: Consumption of Bison from GonaCon Experiment

Hi Jack-

I've been having discussions with EPA and with Montana Department of Agriculture on our proposal to not obtain an Experimental Use Permit for the pesticide-related FIFRA requirements. EPA's generally in agreement that since the study is conducted in a confined area with fences, the EUP isn't necessary. The only remaining issue is that EPA wants us to guarantee that the bison from the experiment won't be consumed. Can we absolutely guarantee this?

From: [Matt McCollum](#)
To: Rebecca.K.Frey@aphis.usda.gov
Cc: [Jack C Rhyan](#); [Patrick R Clarke](#); [Pauline Nol](#)
Subject: Re: contraception editing
Date: Friday, October 08, 2010 11:19:00 AM
Attachments: [ImmunocontBisonProject_mpm.doc](#)

Here are my (very) quick 2 cents.

Matt

(See attached file: ImmunocontBisonProject_mpm.doc)

☐ Rebecca K Frey---10/08/2010 07:49:23 AM---Hello all, Just wanted to remind everyone that we need to keep this ball rolling as quickly as possible. Please have any revi

**Rebecca K
Frey/MT/APHIS/USDA**

10/08/2010 07:49 AM

ToJack C Rhyan/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA, Patrick
R Clarke/MT/APHIS/USDA@USDA

cc

Subjectcontraception editing

Hello all,

Just wanted to remind everyone that we need to keep this ball rolling as quickly as possible. Please have any revisions and clarifications of the contraception study to Jack by COB Friday the 15th (Matt.....you are on the hook for today!!). Jack, can you commit to preparing the final version within a week from that date? Then we can officially ask for the EA to be prepared.

Thanks!

Becky

this is what I had as the last version of this doc. with a couple of my original ?'s

[attachment "ImmunocontBisonProject_rkf.doc" deleted by Matt McCollum/CO/APHIS/USDA]

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425
(b) (6) cell

Proposed Project:

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites

2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

This general research plan will be followed; details will be worked out in further consultation with collaborators and a more extensive protocol developed. A total of approximately 46 yearling bison (approximately half seropositive and half seronegative females and 6 seronegative males) captured in winter/spring as part of the ongoing Interagency Bison Management Plan will be transported to the ~~bison quarantine feasibility study~~ USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Seronegative animals will be separated from seropositives and monitored monthly by serology until August. Bulls will be maintained separately and monitored by serology. In August, animals will be relocated into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive GonaCon™ vaccine and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female sentinel bison will be fitted with proximity collars programmed to record proximity to one another and to transmitters on vaginal implants. Over the 3 year period, calving, abortion results, and serology in the groups will be monitored. In February each year animals will be pregnancy tested and pregnant animals fitted with vaginal transmitters. Transmitters will alert investigators to abortion or calving events and record exposure of sentinel animals. Animals will be tested by serology in February and in summer following calving. At the end of the study, necropsy and culture of all adult animals will occur. Offspring from the study will be monitored by serology and culture twice a year throughout the study. Offspring that remain or become positive for *B. abortus* by serology or culture after weaning will be euthanized and necropsied. Offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be translocated to tribal and/or public lands. Or sold with the proceeds going to fund further research?

Expected outcomes:

Commented [m1]: Are we sticking with yearlings or are we potentially going to get other ages?

Commented [m2]: How important are the collars given the setting of this study? They are all going to be forced into relatively close proximity just by virtue of being in the same fenced pasture. This might be a place to save some \$ without losing much from the overall study.

Commented [rkf3]: Three years from when? After injection; or after 3 possible births. In other words...if we were to get animals in 2011, when would they be necropsied? Is there any need to keep them more than 3 years?

Commented [rkf4]: Had to read twice to comprehend.....all animals from original capture only?

Commented [m5]: Do we want to go down this road again?

1. Determine the effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds.
2. Determine the effect prolonged anestrus has on the transmission of *B. abortus* in bison herds.
3. Determine the risk and extent of exposure of bison herd members to *B. abortus* at parturition sites.
4. Determine nature of infection in calves due to suckling of seropositive cows.
5. Determine risk of venereal transmission of *B. abortus* to seronegative bull bison.

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Coop Agreement draft
Date: Friday, February 15, 2013 11:59:00 AM
Attachments: [APHIS_BisonTransferAgreementJRedits_Feb2013\(1\)ON edits.docx](#)

[Made some edits...](#)

From: Rhyan, Jack C - APHIS
Sent: Friday, February 15, 2013 10:58 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: Coop Agreement draft
How's this?
Jack

INTERAGENCY AGREEMENT
between the
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
and the
NATIONAL PARK SERVICE

ARTICLE I. BACKGROUND AND OBJECTIVES

To evaluate sterilization by use of GonaCon™, an immunocontraceptive vaccine, ~~and ovariectomy~~ as means of decreasing the potential for transmission of *Brucella abortus* in bison. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

ARTICLE II. STATEMENT OF WORK

A. During the period of performance, up to 63 live bison (8-16 seronegative bulls, 32-40 seropositive cows, 5-7 seronegative cows) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to determine whether:

- Immunocontraception ~~and/or ovariectomy procedures~~ can prevent the shedding of *Brucella abortus* bacteria in young, recently infected bison;
- Immunocontraception with GonaCon™ vaccine can prevent shedding of *Brucella abortus* bacteria throughout the infection cycle;
- Recovery from the contraceptive treatment and the brucellosis infection can be completed without any further shedding of the bacteria during subsequent pregnancies; and
- ~~Behavioral changes occur during the breeding season when females are treated with two types of pregnancy prevention procedures.~~

~~Any bulls that seroconvert to positive may, with notification of the NPS Key Official, be transferred to an APHIS quarantine facility in Fort Collins, CO, for a venereal transmission study.~~

B. Any bulls that seroconvert to positive may, with notification of the NPS Key Official, be transferred to an APHIS quarantine facility in Fort Collins, CO, for a venereal transmission study.

BC. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

CD. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

DE. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

ARTICLE III. TERM OF AGREEMENT

The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

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ARTICLE IV. KEY OFFICIALS

National Park Service
Yellowstone Center for Resources
Rick Wallen, Wildlife Biologist
P.O. Box 168
Yellowstone National Park, WY 82190
307-344-2285
rick_wallen@nps.gov

Animal and Plant Health Inspection Service
Veterinary Services
Jack Rhyan, DVM
National Wildlife Research Center
Fort Collins, CO 80521
970-266-6140
Jack.C.Rhyan@aphis.usda.gov

ARTICLE V. PAYMENT

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status as described in OA 1858– “Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison” and the Environmental Assessment – “Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison in the Greater Yellowstone Area” (USDA, May 2012):- “At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses from non-vaccinated bison will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.”

~~Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure will should be:~~

- ~~consigned to a quarantine location for further diagnostics, directly~~

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Commented [pn1]: I guess this is not the quote in the protocol but I guess we wouldn't want to make it look like vaccinated animals will go into the food chain. Maybe state this somewhere else?

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- ~~to be consigned to~~ a managed for public trust conservation program to supplement population genetic diversity
- ~~to be consigned to~~ an introduction program to establish a new conservation population of wild bison ~~on tribal or public lands~~, or
- ~~utilized in an embryo transfer program for bison genetics conservation.~~

~~If no such opportunities exist, bison will be consigned to a private not-for-profit bison conservation program, or as a last choice, to. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis free bison to~~ any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities ~~in Corwin Springs, MT, or Fort Collins, CO, for this research~~ and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

ARTICLE VII. PRIOR APPROVAL

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq., 16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in ~~the same research study~~ at the ~~same above specified~~ locations.

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The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

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Either party may terminate the agreement by providing 14 days advance written notice to the other party.

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IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

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Signature: _____
Name: Daniel N. Wenk
Title: Superintendent, Yellowstone NP
Date: February ____, 2013

Signature: _____
Name: ?????????? Mark Davidson
Title: ?????????? Director,
Western Region, USDA, APHIS, VS
Date: February ____, 2013

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Signature: _____
Name: Tina Holland
Title: Contracting Officer
Date: February ____, 2013

Signature: _____
Name: ??????????
Title: ??????????
Date: February ____, 2013

From: [Nol, Pauline - APHIS](#)
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Rick Wallen, Wildlife Biologist
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307-344-2285
rick_wallen@nps.gov

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Signature: _____
Name: Daniel N. Wenk
Title: Superintendent, Yellowstone NP
Date: February ____, 2013

Signature: _____
Name: 999999999 Mark Davidson
Title: 999999999 Director,
Western Region, USDA, APHIS, VS
Date: February ____, 2013

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Signature: _____
Name: Tina Holland
Title: Contracting Officer
Date: February ____, 2013

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Name: 999999999
Title: 999999999
Date: February ____, 2013

From: [Clarke, Patrick R. - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Coop Agreement draft
Date: Friday, February 15, 2013 2:27:06 PM
Attachments: [APHIS_BisonTransferAgreementJRedits_Feb2013\(1\)ON--PRC edits.docx](#)

Made a few more.....

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Nol, Pauline - APHIS
Sent: Friday, February 15, 2013 11:59 AM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Subject: RE: Coop Agreement draft
[Made some edits...](#)

From: Rhyan, Jack C - APHIS
Sent: Friday, February 15, 2013 10:58 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: Coop Agreement draft
How's this?
Jack

INTERAGENCY AGREEMENT
between the
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
and the
NATIONAL PARK SERVICE

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To evaluate sterilization by use of GonaCon™, an immunocontraceptive vaccine, ~~and ovariectomy~~ as means of decreasing the potential for transmission of *Brucella abortus* in bison. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

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ARTICLE IV. KEY OFFICIALS

National Park Service
Yellowstone Center for Resources
Rick Wallen, Wildlife Biologist
P.O. Box 168
Yellowstone National Park, WY 82190
307-344-2285
rick_wallen@nps.gov

Animal and Plant Health Inspection Service
Veterinary Services
Jack Rhyan, DVM
National Wildlife Research Center
Fort Collins, CO 80521
970-266-6140
Jack.C.Rhyan@aphis.usda.gov

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Date: February ____, 2013

Signature: _____
Name: ???????????
Title: ???????????
Date: February ____, 2013


From: [Jack C Rhyan](#)
To: [Patrick R Clarke](#); [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: Discussion with John Clifford, decreasing wildlife disease prevalence
Date: Thursday, February 11, 2010 3:56:00 PM

I sent this to Brian and forgot to cc y'all.

Jack

Thanks, Brian. We'll draft a proposal with budget for you.

Jack

 [Brian J McCluskey](#)---02/11/2010 02:51:08 PM---Hey everyone, I discussed our intention to focus our efforts primarily on the reduction of prevalence in wildlife with Dr. Clif

**Brian J
McCluskey/CO/APHIS/USDA**

02/11/2010 02:51 PM

ToJack C
Rhyan/CO/APHIS/USDA@USDA,
Patrick R
Clarke/MT/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA,
Rebecca K
Frey/MT/APHIS/USDA@USDA

cc

SubjectDiscussion with John Clifford, decreasing
wildlife disease prevalence

Hey everyone,

I discussed our intention to focus our efforts primarily on the reduction of prevalence in wildlife with Dr. Clifford and he is supportive. No need for major announcement. In future projects, presentations, discussions, etc. remember that this our focus and take the opportunity to remind stakeholders that the disease is only in the wildlife and we need to reduce prevalence in those populations. Given this is the focus the idea of the immunocontraception study is a good one. We need a proposal for this.

Brian

Brian J. McCluskey, DVM, PhD, Dip. ACVPM
Director, Veterinary Services, Western Region
Fort Collins, CO
970.494.7385

From: [Patrick R Clarke](#)
To: [Rebecca K Frey](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Pauline Nol](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 10:15:00 AM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.13.11mm_pn prc comments.docx](#)

I second that emotion.

My two cent version/comments

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.13.11mm_pn prc comments.docx)

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell

☐ Rebecca K Frey---05/13/2011 09:34:12 AM---also....this version still says December for bulls out.....I refuse to calve for 6 months..... :-)

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
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Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

☐ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

05/13/2011 09:10 AM

To: Rebecca K Frey/MT/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subject: Re: draft ACUC protocol



Latest version. Jack pointed out that I forgot the replicate-so there will be 92 cows collected over 3-4 years.

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx" deleted by Rebecca K Frey/MT/APHIS/USDA]

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

☐ Rebecca K Frey---05/12/2011 04:10:08 PM---Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include al

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Date: 05/12/2011 04:10 PM
Subject: Re: draft ACUC protocol

Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include all of them.
Becky Frey


☐ Pauline Nol---05/12/2011 02:28 PM MDT---Thank you Becky for your comments! That first table with all the questions about regulatory consider

From: Pauline Nol
To: Rebecca Frey
Cc: Jack Rhyan; Matt McCollum; Patrick Clarke
Date: 05/12/2011 02:28 PM MDT
Subject: Re: draft ACUC protocol

Thank you Becky for your comments! That first table with all the questions about regulatory considerations may not even need to be in the document. I just hadn't taken it out. None of that information is requested by the CSU IACUC for instance.

I changed the bull exposure to October. And everyone had a question about who we will euthanize at the end of the study (as long as they are not fatally injured). I was originally thinking that the few sentinels should be euthanized at the end for culture purposes whether they are seropositive or not. But I suppose we have plenty of data showing correlation between seroconversion and culture positivity, as we talked about the other day. Thoughts?

P

 Rebecca K Frey---05/12/2011 02:04:54 PM---Just put a few questions/comments in.....Nice! Rebecca Frey, Wildlife Biologist/Disease Specialist

From: Rebecca K Frey/MT/APHIS/USDA
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Just put a few questions/comments in.....Nice!

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx" deleted by Pauline Nol/CO/APHIS/USDA]

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ <u>YELL-2011-SCI-5892</u> _____ <u>May 10, 2011</u> Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
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<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
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Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

Commented [pn1]:

DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator Attending Veterinarian
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

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2. Testing Facilities

Name	Address	Role in Study
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USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

Commented [r2]: I could probably talk Marty Z into paying for the serology at the DOL lab

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2017
Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

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Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 46 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 10 seronegative and 36 seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, and 2013 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Commented [pn3]: Only semi-annually?

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. If not enough animals are collected by spring 2012 then two test pastures will be established in 2012 and two test pastures in 2013. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Commented [r4]: do we want to put extra seronegatives in each group in April 2012 to compensate for any seroconverting between April (selected into groups) and August? Like 6 negs?

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [r5]: I don't think we need to monitor it. Are we trying to mimic "wild" conditions where the predators would have it gone within by 24 hours?

Commented [pn6]: Do we want to do this?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [mm7]: If we do do this, is this where we set up cameras?

Commented [pn8]: Do we want to do this more to monitor trends in serology (vax vs nonvax)?

At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [mm9]: seropositive?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions).

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 46 females, 4 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be ~~trapped~~ captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

Commented [r10]: Trapped isn't PC

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be ~~trapped~~ captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either performed by trained personnel using a captive bolt, a bullet from a shot with a high powered rifle, or appropriate chemical euthanasia solutions ~~administered~~ immobilized and administered, as A animals will be chemically immobilized prior to euthanasia, ~~or 88 mg/kg when appropriate, pentobarbital, as the situation requires.~~ The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than

momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Dr. P. Ryan Clarke

Date of Consultation: 13 May 21011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. ~~be administered either by captive bolt, shot with a high-powered rifle, or chemically immobilized and administered, as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.~~

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

- Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80
- Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30
- Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.
- Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?
<input checked="" type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe species, potential impact and measures to be taken to minimize impact:
Consultations:
Did you consult with a state or federal agency specifically on this action.
<input type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe the date/mode/contact person and outcome of this consultation:
Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.
<input type="checkbox"/> No, permission not needed because:
<input type="checkbox"/> Yes

PART ONE: SIGNATURE PAGE

Study Director: _____

Date: _____

Concur:
IACUC Chair _____ Date _____

From: [Pauline Nol](#)
To: [Matt McCollum](#)
Cc: [Jack C Rhyan](#); [Patrick R Clarke](#); [Rebecca K Frey](#)
Subject: RE: draft ACUC protocol
Date: Thursday, May 12, 2011 2:17:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.12.11mm_pn comments.docx](#)

Hi,
Here is a better version. It actually has a title now!

Thanks for your comments, Matt, especially for pointing out that a fatal injury means already dead!

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11mm_pn comments.docx)

 [Matt McCollum](#)---05/12/2011 01:57:29 PM---Looks good Pauline, I made a few suggestions and asked some questions.

From: Matt McCollum/CO/APHIS/USDA@MSOCOEX
To: Pauline Nol/CO/APHIS/USDA, Jack C Rhyan/CO/APHIS/USDA, Rebecca K Frey/MT/APHIS/USDA, Patrick R Clarke/MT/APHIS/USDA
Date: 05/12/2011 01:57 PM
Subject: RE: draft ACUC protocol

Looks good Pauline,

I made a few suggestions and asked some questions.

Matt

From: Nol, Pauline (APHIS)
Sent: Thursday, May 12, 2011 11:58 AM
To: Rhyan, Jack C (APHIS); McCollum, Matthew P (APHIS); Frey, Rebecca K (APHIS); Clarke, Ryan P. (APHIS)
Subject: draft ACUC protocol

Hi guys,
Did this **real quick** hijacking another template. It may have stray "NWRC's" in places where I forgot to delete them.
And I don't have the references in place yet.
Let me know what you think so far. And I will come back to it later this afternoon to go through it better with an editing cap.
Pauline

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11.docx)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138

pauline.nol@aphis.usda.gov[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5
12 11mpm.docx" deleted by Pauline Nol/CO/APHIS/USDA]

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.
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Commented [pn1]:

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Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

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	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
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Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
------	---------	---------------

USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2017
Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

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Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 46 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 10 seronegative and 36 seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, and 2013 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Commented [pn2]: Only semi-annually?

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. If not enough animals are collected by spring 2012 then two test pastures will be established in 2012 and two test pastures in 2013. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [pn3]: Do we want to do this?

Commented [mm4]: If we do do this, is this where we set up cameras?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [pn5]: Do we want to do this more to monitor trends in serology (vax vs nonvax)?

At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [mm6]: seropositive?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions).

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 46 females, 4 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered, as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: _____

Date of Consultation: _____

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered, as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

PART ONE: SIGNATURE PAGE

Study Director: _____

Date: _____

Concur:
IACUC Chair _____ Date _____

From: [Matt McCollum](#)
To: [Rebecca K Frey](#); [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Patrick R Clarke](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 9:37:00 AM

Suck it up Buttercup

From: Frey, Rebecca K (APHIS)
Sent: Friday, May 13, 2011 09:34 AM
To: Nol, Pauline (APHIS)
Cc: Rhyan, Jack C (APHIS); McCollum, Matthew P (APHIS); Clarke, Ryan P. (APHIS)
Subject: Re: draft ACUC protocol

also....this version still says December for bulls out.....I refuse to calve for 6 months..... :-)

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

▼ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

05/13/2011 09:10 AM

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

SubjectRe: draft ACUC protocol📎

Latest version. Jack pointed out that I forgot the replicate-so there will be 92 cows collected over 3-4 years.
[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx" deleted by Rebecca K Frey/MT/APHIS/USDA]

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

▼ Rebecca K Frey---05/12/2011 04:10:08 PM---Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include al

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Date: 05/12/2011 04:10 PM
Subject: Re: draft ACUC protocol

Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include all of them.
Becky Frey

▼ Pauline Nol---05/12/2011 02:28 PM MDT---Thank you Becky for your comments! That first table with all the questions about regulatory consider

From: Pauline Nol
To: Rebecca Frey
Cc: Jack Rhyan; Matt McCollum; Patrick Clarke
Date: 05/12/2011 02:28 PM MDT
Subject: Re: draft ACUC protocol

Thank you Becky for your comments! That first table with all the questions about regulatory considerations may not even need to be in the document. I just hadn't taken it out. None of that information is requested by the CSU IACUC for instance.

I changed the bull exposure to October. And everyone had a question about who we will euthanize at the end of the study (as long as they are not fatally injured). I was originally thinking that the few sentinels should be euthanized at the end for culture purposes whether they are seropositive or not. But I suppose we have plenty of data showing correlation between seroconversion and culture positivity, as we talked about the other day. Thoughts?
P

▼ Rebecca K Frey---05/12/2011 02:04:54 PM---Just put a few questions/comments in.....Nice! Rebecca Frey, Wildlife Biologist/Disease Specialist

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 02:04 PM
Subject: Re: draft ACUC protocol

Just put a few questions/comments in.....Nice!

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx" deleted by Pauline

Nol/CO/APHIS/USDA]

Rebecca Frey, Wildlife Biologist/Disease Specialist

USDA APHIS VS

Emigrant, Montana

(406) 333-4425

From: [Matt McCollum](#)
To: [Pauline Nol](#); [Jack C Rhyan](#); [Rebecca K Frey](#); [Patrick R Clarke](#)
Subject: RE: draft ACUC protocol
Date: Thursday, May 12, 2011 1:57:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.12.11mpm.docx](#)

Looks good Pauline,

I made a few suggestions and asked some questions.

Matt

From: Nol, Pauline (APHIS)
Sent: Thursday, May 12, 2011 11:58 AM
To: Rhyan, Jack C (APHIS); McCollum, Matthew P (APHIS); Frey, Rebecca K (APHIS); Clarke, Ryan P. (APHIS)
Subject: draft ACUC protocol

Hi guys,
Did this **real quick** hijacking another template. It may have stray "NWRC's" in places where I forgot to delete them.

And I don't have the references in place yet.
Let me know what you think so far. And I will come back to it later this afternoon to go through it better with an editing cap.
Pauline

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11.docx)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
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1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services

Study Title:	
Study Director:	
:	

REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ Pending _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

Commented [pn1]:

Commented [pn2]:

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

PART FOUR: STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator

2. Testing Facilities

Name	Address	Role in Study
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Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

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Proposed Experimental Termination Date: October 1, 2017
Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

Commented [mm3]: Calves?

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *B. abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. ~~immunocontraceptive~~ Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

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8. Methods/Procedures

A total of 46 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 10 seronegative and 36 seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, and 2013 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Commented [mm4]: Does this mean every two months or twice a month?

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. If not enough animals are collected by spring 2012 then two test pastures will be established and in 2012 and two test pastures in 2013. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [pn5]: Do we want to do this?

Commented [mm6]: If we do do this, is this where we set up cameras?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [pn7]: Do we want to do this more to monitor trends in serology (vax vs nonvax)?

At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [mm8]: seropositive?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed Brucella if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have [aan](#) 82% power to detect a 23% change (30% to 7% abortions).

10. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 46 females, 4 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species

4) Source: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification

6) Trapping/Collecting: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10). Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered, ~~as animals will be chemically immobilized prior to euthanasia, or~~ 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

Commented [mm9]: Wouldn't it already be dead? Mortally? Significantly? Badly?

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

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11) Animal pain or distress

1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: _____

Date of Consultation: _____

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

M. Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

Commented [mm10]: Same as above I would like to keep it open for all possibilities depending on the situation

N. IACUC Approval

Date of IACUC Approval Letter: _____

O. Staff Qualifications

18. References

Manthei et al., 1950;
Rankin, 1965),
Robison et al., 1998
Miller LA, Rhyon JC, and Drew, M, 2004

Commented [pn11]: Still need to write these out

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chemo.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?
<input checked="" type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe species, potential impact and measures to be taken to minimize impact:
Consultations:
Did you consult with a state or federal agency specifically on this action.
<input type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe the date/mode/contact person and outcome of this consultation:
Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.
<input type="checkbox"/> No, permission not needed because:
<input type="checkbox"/> Yes

Commented [pn12]:

Commented [pn13]:

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/KLH Conjugate (1000 µg)

Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
<i>Concholepas concholepas</i> hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml

AdjuVac™ adjuvant

<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

Commented [pn14]: ??

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

Commented [pn15]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____Concur:
NWRC Research Project Leader _____ Date _____Review and Processing:
QAU: _____ Date _____Concur:
NWRC Assistant Director _____ Date _____Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

From: [Rebecca K Frey](#)
To: [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Patrick R Clarke](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 9:34:00 AM

also....this version still says December for bulls out.....I refuse to calve for 6 months..... :-)

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

☐ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

05/13/2011 09:10 AM

To: Rebecca K Frey/MT/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subject: Re: draft ACUC protocol



Latest version. Jack pointed out that I forgot the replicate-so there will be 92 cows collected over 3-4 years.

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx" deleted by Rebecca K Frey/MT/APHIS/USDA]

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
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Fort Collins, CO 80521
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Cell: (b) (6)
Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

☐ Rebecca K Frey---05/12/2011 04:10:08 PM---Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include al

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To: Pauline Nol/CO/APHIS/USDA@USDA
Date: 05/12/2011 04:10 PM
Subject: Re: draft ACUC protocol

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Becky Frey

☐ Pauline Nol---05/12/2011 02:28 PM MDT---Thank you Becky for your comments! That first table with all the questions about regulatory consider

From: Pauline Nol
To: Rebecca Frey
Cc: Jack Rhyan; Matt McCollum; Patrick Clarke
Date: 05/12/2011 02:28 PM MDT
Subject: Re: draft ACUC protocol

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I changed the bull exposure to October. And everyone had a question about who we will euthanize at the end of the study (as long as they are are not fatally injured). I was originally thinking that the few sentinels should be euthanized at the end for culture purposes whether they are seropositive or not. But I suppose we have plenty of data showing correlation between seroconversion and culture positivity, as we talked about the other day. Thoughts?

P

☐ Rebecca K Frey---05/12/2011 02:04:54 PM---Just put a few questions/comments in.....Nice! Rebecca Frey, Wildlife Biologist/Disease Specialist

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 02:04 PM
Subject: Re: draft ACUC protocol

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[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx" deleted by

Pauline Nol/CO/APHIS/USDA]

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

From: [Rebecca K Frey](#)
To: [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Patrick R Clarke](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 9:29:00 AM

In that case, I think we should take the dates out, since they will be born in a lot of different years, stick to ages. ie. 1,2, and 3 yo.

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

☐ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

05/13/2011 09:10 AM

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subject

Re: draft ACUC protocol



Latest version. Jack pointed out that I forgot the replicate-so there will be 92 cows collected over 3-4 years.

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Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

From: [Pauline Nol](#)
To: [Rebecca K Frey](#); [Patrick R Clarke](#); [Jack C Rhyan](#); [Matt McCollum](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 9:10:00 AM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx](#)

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(See attached file: *ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx*)

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Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

Permits	
<input type="checkbox"/>	<input checked="" type="checkbox"/>
Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. National Park Service <u>YELL-2011-SCI-5892</u> <u>May 10, 2011</u> Permit(s) description Number Date	
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)	
<input type="checkbox"/>	<input checked="" type="checkbox"/>
Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .	
<input checked="" type="checkbox"/>	<input type="checkbox"/>
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<input type="checkbox"/>	<input checked="" type="checkbox"/>
Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.	
<input checked="" type="checkbox"/>	<input type="checkbox"/>
Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.	
Regulatory Standard and Test Guidelines	

Commented [pn1]:

DESCRIPTION OF ACTIVITIES

Nature of the Collaboration:	<input type="checkbox"/> Advisory Committee participation															
	<input checked="" type="checkbox"/> Manuscript/review article collaboration															
	<input type="checkbox"/> Training program requiring the use of animals															
	<input checked="" type="checkbox"/> Data analysis, interpretation and reporting															
	<input checked="" type="checkbox"/> Other: <u>Live animal work</u>															
Collaboration:	<table border="1"> <thead> <tr> <th>Name</th> <th>Address or Organization</th> <th>Role in Project</th> </tr> </thead> <tbody> <tr> <td>Jack Rhyan</td> <td>USDA, APHIS, VS</td> <td>Principle Investigator</td> </tr> <tr> <td>Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum</td> <td>USDA, APHIS, VS</td> <td>Investigators</td> </tr> <tr> <td>Rick Wallen, Jenny Powers</td> <td>National Park Service</td> <td>Investigators</td> </tr> <tr> <td>Lowell Miller, Kathy Fagerstone</td> <td>USDA, APHIS, WS, National Wildlife Research Center</td> <td>Investigators</td> </tr> </tbody> </table>	Name	Address or Organization	Role in Project	Jack Rhyan	USDA, APHIS, VS	Principle Investigator	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators	Rick Wallen, Jenny Powers	National Park Service	Investigators	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators
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Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators														

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
 Proposed Experimental Termination Date: October 1, 2017
 Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison

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3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

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8. Methods/Procedures

A total of ~~9246~~ female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately ~~240~~ seronegative and ~~7236~~ seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, ~~and 2013~~, and 2014 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Commented [pn2]: Only semi-annually?

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. ~~If not enough animals are collected by spring 2012 then t~~Two test pastures will be established in 2012 and two test pastures in 2013 or 2014 if not enough animals will be captured. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [pn3]: Do we want to do this?

Commented [mm4]: If we do do this, is this where we set up cameras?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [pn5]: Do we want to do this more to monitor trends in serology (vax vs nonvax)

At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [mm6]: seropositive?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA
Total Number and Sex: 46 females, 4 males
Body weight range: 400-1000 kg
Age: 2 year to adult

- 2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.
- 3) Rationale for appropriateness of the species to be used: Bison are the target species.
- 4) Source: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.
- 6) Trapping/Collecting: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.
- 8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.
- 9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:
Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: _____

Date of Consultation: _____

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered 88 mg/kg pentobarbital, as the situation requires. The carcasses of

ethanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Concur:
IACUC Chair _____ Date _____

From: [Rebecca K Frey](#)
To: [Pauline Nol](#)
Subject: Re: draft ACUC protocol
Date: Thursday, May 12, 2011 4:10:00 PM

Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include all of them.

Becky Frey


 Pauline Nol---05/12/2011 02:28 PM MDT---Thank you Becky for your comments! That first table with all the questions about regulatory consider

From: Pauline Nol
To: Rebecca Frey
Cc: Jack Rhyan; Matt McCollum; Patrick Clarke
Date: 05/12/2011 02:28 PM MDT
Subject: Re: draft ACUC protocol

Thank you Becky for your comments! That first table with all the questions about regulatory considerations may not even need to be in the document. I just hadn't taken it out. None of that information is requested by the CSU IACUC for instance.

I changed the bull exposure to October. And everyone had a question about who we will euthanize at the end of the study (as long as they are are not fatally injured). I was originally thinking that the few sentinels should be euthanized at the end for culture purposes whether they are seropositive or not. But I suppose we have plenty of data showing correlation between seroconversion and culture positivity, as we talked about the other day. Thoughts?

P

 Rebecca K Frey---05/12/2011 02:04:54 PM---Just put a few questions/comments in.....Nice! Rebecca Frey, Wildlife Biologist/Disease Specialist

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 02:04 PM
Subject: Re: draft ACUC protocol

Just put a few questions/comments in.....Nice!

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx" deleted by Pauline Nol/CO/APHIS/USDA]


Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

From: [Pauline Nol](#)
To: [Rebecca K Frey](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Patrick R Clarke](#)
Subject: Re: draft ACUC protocol
Date: Thursday, May 12, 2011 2:28:00 PM

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To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 02:04 PM
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Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

From: [Rebecca K Frey](#)
To: [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Patrick R Clarke](#)
Subject: Re: draft ACUC protocol
Date: Thursday, May 12, 2011 2:04:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.12.11\(rkf\).docx](#)

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(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx)

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services

Study Title:	
Study Director:	
:	

REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. <div style="display: flex; justify-content: space-between;"> <div>National Park Service</div> <div>Pending</div> </div> <div style="display: flex; justify-content: space-between;"> <div>Permit(s) description</div> <div>Number</div> <div>Date</div> </div>
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
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<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

Commented [pn1]:

Commented [pn2]:

Commented [rkf3]: Which are you referring to here? Possible future movement?

Commented [rkf4]: Does the transfer of tissues to the labs meet this definition?

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

PART FOUR: STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator

Commented [rkf5]: Do we need to add Jason L?

2. Testing Facilities

Name	Address	Role in Study
------	---------	---------------

USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2017
Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *B. abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 46 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 10 seronegative and 36 seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, and 2013 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. If not enough animals are collected by spring 2012 then two test pastures will be established and in 2012 and two test pastures in 2013. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

Commented [rkf6]: Can we make this October so we tighten up calving?

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [pn7]: Do we want to do this?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [pn8]: Do we want to do this more to monitor trends in serology (vax vs nonvax)

At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [rkf9]: Is this just the vaccinates?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed Brucella if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have a 82% power to detect a 23% change (30% to 7% abortions).

10. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 46 females, 4 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species

4) Source: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification

6) Trapping/Collecting: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10). Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than

momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: _____

Date of Consultation: _____

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

M. Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter: _____

O. Staff Qualifications

18. References

Manthei et al., 1950;
Rankin, 1965),
Robison et al., 1998
Miller LA, Rhyon JC, and Drew, M, 2004

Commented [pn10]: Still need to write these out

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?
<input checked="" type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe species, potential impact and measures to be taken to minimize impact:
Consultations:
Did you consult with a state or federal agency specifically on this action.
<input type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe the date/mode/contact person and outcome of this consultation:
Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.
<input type="checkbox"/> No, permission not needed because:
<input type="checkbox"/> Yes

Commented [pn11]:

Commented [pn12]:

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/KLH Conjugate (1000 µg)

Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
<i>Concholepas concholepas</i> hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml

AdjuVac™ adjuvant

<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

Commented [pn13]: ??

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

Commented [pn14]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____Concur:
NWRC Research Project Leader _____ Date _____Review and Processing:
QAU: _____ Date _____Concur:
NWRC Assistant Director _____ Date _____Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

From: [Patrick R Clarke](#)
To: [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Matt McCollum](#); [Rebecca K Frey](#)
Subject: Re: draft ACUC protocol
Date: Thursday, May 12, 2011 1:36:00 PM

I have sent this out to Dan Tyres (has committed to stay on the ACUC), to Tim Griffiths (who I haven't been able to talk to directly since he has been on leave), and Jerry Wiscomb (our committee candidate if Tim ops out)

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell

 Pauline Nol---05/12/2011 11:57:57 AM---Hi guys, Did this real quick hijacking another template. It may have stray "NWRC's" in places where

From: Pauline Nol/CO/APHIS/USDA
To: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Rebecca K Frey/MT/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 11:57 AM
Subject: draft ACUC protocol

Hi guys,
Did this **real quick** hijacking another template. It may have stray "NWRC's" in places where I forgot to delete them.
And I don't have the references in place yet.
Let me know what you think so far. And I will come back to it later this afternoon to go through it better with an editing cap.
Pauline

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11.docx" deleted by Patrick R Clarke/MT/APHIS/USDA]

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521

Ph: (970) 266-6126

Cell: (b) (6)

Fax: (970) 266-6138

pauline.nol@aphis.usda.gov

From: [Pauline Nol](#)
To: [Patrick R Clarke](#)
Cc: [Rebecca K Frey](#); [Jack C Rhyan](#); [Matt McCollum](#)
Subject: Re: draft ACUC protocol
Date: Friday, May 13, 2011 1:34:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.13.11 \(2\).docx](#)

Okay,

I think I got all the changes and comments in one draft now. Becky, I **promise** I changed the December to October in a previous version, but I guess fate wants it to be December. No, it really is changed now. There were a few discussion points that I left hanging, ie what to do with aborted fetuses. I took the NEPA part out including the appendix. Probably don't need that complicating ACUC matters. I put Jason in as an investigator. Should we put in Brandt Schumacher?

Change as desired and I guess send on to Jason and then the rest of the gang.

P

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.13.11 (2).docx)

☐ Patrick R Clarke---05/13/2011 10:15:11 AM---I second that emotion. My two cent version/comments

From: Patrick R Clarke/MT/APHIS/USDA
To: Rebecca K Frey/MT/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA
Date: 05/13/2011 10:15 AM
Subject: Re: draft ACUC protocol

I second that emotion.

My two cent version/comments

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.13.11mm_pn prc comments.docx" deleted by Pauline Nol/CO/APHIS/USDA]

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS

Regional Epidemiologist- GYA
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(406) 388-5162
(b) (6)-cell

☐ Rebecca K Frey---05/13/2011 09:34:12 AM---also....this version still says December for bulls out....I refuse to calve for 6 months..... :-)

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R Clarke/MT/APHIS/USDA@USDA
Date: 05/13/2011 09:34 AM
Subject: Re: draft ACUC protocol

also....this version still says December for bulls out....I refuse to calve for 6 months..... :-)

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

☐ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

05/13/2011 09:10 AM

To: Rebecca K Frey/MT/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Jack C Rhyan/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

cc

Subject: Re: draft ACUC protocol



Latest version. Jack pointed out that I forgot the replicate-so there will be 92 cows collected over 3-4 years.

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.13.11.docx" deleted by Rebecca K Frey/MT/APHIS/USDA]

Pauline Nol, DVM, MS, PhD
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Fax: (970) 266-6138
pauline.nol@aphis.usda.gov

☐ Rebecca K Frey---05/12/2011 04:10:08 PM---Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include al

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA
Date: 05/12/2011 04:10 PM
Subject: Re: draft ACUC protocol

Ya, I think we can spare anybody that has not seroconverted by end of study, but that may include all of them.
Becky Frey

☐ Pauline Nol---05/12/2011 02:28 PM MDT---Thank you Becky for your comments! That first table with all the questions about regulatory considere

From: Pauline Nol
To: Rebecca Frey
Cc: Jack Rhyan; Matt McCollum; Patrick Clarke
Date: 05/12/2011 02:28 PM MDT
Subject: Re: draft ACUC protocol

Thank you Becky for your comments! That first table with all the questions about regulatory considerations may not even need to be in the document. I just hadn't taken it out. None of that information is requested by the CSU IACUC for instance.

I changed the bull exposure to October. And everyone had a question about who we will euthanize at the end of the study (as long as they are are not fatally injured). I was originally thinking that the few sentinels should be euthanized at the end for culture purposes whether they are seropositive or not. But I suppose we have plenty of data showing correlation between seroconversion and culture positivity, as we talked about the other day. Thoughts?
P

☐ Rebecca K Frey---05/12/2011 02:04:54 PM---Just put a few questions/comments in.....Nice! Rebecca Frey, Wildlife Biologist/Disease Specialist

From: Rebecca K Frey/MT/APHIS/USDA
To: Pauline Nol/CO/APHIS/USDA@USDA

Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Patrick R
Clarke/MT/APHIS/USDA@USDA
Date: 05/12/2011 02:04 PM
Subject: Re: draft ACUC protocol

Just put a few questions/comments in.....Nice!

[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5.12.11(rkf).docx" deleted by
Pauline Nol/CO/APHIS/USDA]

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.
		<div> <div>National Park Service</div> <div>YELL-2011-SCI-5892</div> <div>May 10, 2011</div> </div>
		<div> <div>Permit(s) description</div> <div>Number</div> <div>Date</div> </div>

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ Advisory Committee participation
 - ☒ Manuscript/review article collaboration
 - ☐ Training program requiring the use of animals
 - ☒ Data analysis, interpretation and reporting
 - ☒ Other: Live animal work

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, <u>Jason Lombard</u>	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator

Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator/Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

Commented [r1]: I could probably talk Marty Z into paying for the serology at the DOL lab

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019
October 1, 2018

Commented [pn2]: This would accommodate 5 years of observations if the replicate isn't started until 2014

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle

(Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyon JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/V/S bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute.

Commented [pn3]: I added more cows to allow for seroconverters so there will be 4-6 seronegative sentinels per pen (4 pens total)

Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of ~~four approximately pastures~~ approximately 23 acres each. Each pasture will contain 16-18 seropositives ~~cows~~ and 4-6 seronegatives and 2 bulls. ~~Two replicate~~ test pastures will be established in ~~spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive~~ bison in ~~one pastures~~ will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining ~~two pastures~~ will not be vaccinated.

Commented [r4]: do we want to put extra seronegatives in each group in April 2012 to compensate for any seroconverting between April (selected into groups) and August? Like 6 negs?

Bulls will be separated from the cows outside of breeding season, from ~~December-October~~ until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

~~Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals.~~ The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [r5]: I don't think we need to monitor it. Are we trying to mimic "wild" conditions where the predators would have it gone within by 24 hours?

Commented [pn6]: Do we want to do this?

Commented [mm7]: If we do do this, is this where we set up cameras?

Commented [pn8]: Do we want to do this more to monitor trends in serology (vax vs nonvax)?

In addition, serology for each of the cows, bulls, and calves will be monitored ~~twice a year~~. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all ~~seropositive adult~~ animals will be euthanized and necropsied with specimens collected for culture. ~~The carcasses will be donated to local food banks or Indian tribes.~~ Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 46 females, 4 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Rhyan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Concur:
IACUC Chair _____ Date _____

From: [Matt McCollum](#)
To: [Pauline Nol](#)
Cc: [Jack C Rhyan](#); [Patrick R Clarke](#); [Rebecca K Frey](#)
Subject: RE: draft ACUC protocol
Date: Thursday, May 12, 2011 2:31:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.12.11mpm.docx](#)

I re-refixed the euthanasia part and took out the QA number in the header. I was going to look over the signature page, but you beat me to it.

M

From: Nol, Pauline (APHIS)
Sent: Thursday, May 12, 2011 2:18 PM
To: McCollum, Matthew P (APHIS)
Cc: Rhyan, Jack C (APHIS); Clarke, Ryan P. (APHIS); Frey, Rebecca K (APHIS)
Subject: RE: draft ACUC protocol

Hi,
Here is a better version. It actually has a title now!

Thanks for your comments, Matt, especially for pointing out that a fatal injury means already dead!

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11mm_pn comments.docx)

▼ [Matt McCollum---05/12/2011 01:57:29 PM---](#)Looks good Pauline, I made a few suggestions and asked some questions.

From: Matt McCollum/CO/APHIS/USDA@MSOCOEX
To: Pauline Nol/CO/APHIS/USDA, Jack C Rhyan/CO/APHIS/USDA, Rebecca K Frey/MT/APHIS/USDA, Patrick R Clarke/MT/APHIS/USDA
Date: 05/12/2011 01:57 PM
Subject: RE: draft ACUC protocol

Looks good Pauline,

I made a few suggestions and asked some questions.

Matt

From: Nol, Pauline (APHIS)
Sent: Thursday, May 12, 2011 11:58 AM
To: Rhyan, Jack C (APHIS); McCollum, Matthew P (APHIS); Frey, Rebecca K (APHIS); Clarke, Ryan P. (APHIS)
Subject: draft ACUC protocol

Hi guys,

Did this **real quick** hijacking another template. It may have stray "NWRC's" in places where I forgot to delete them.

And I don't have the references in place yet.

Let me know what you think so far. And I will come back to it later this afternoon to go through it better with an editing cap.

Pauline

(See attached file: ACUC Proposal GonaConBisonStudy2011 draft 5.12.11.docx)

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-Western Region

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Ph: (970) 266-6126

Cell (b) (6)

Fax:(970) 266-6138

pauline.nol@aphis.usda.gov[attachment "ACUC Proposal GonaConBisonStudy2011 draft 5 12 11mpm.docx"
deleted by Pauline Nol/CO/APHIS/USDA]

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ <u>YELL-2011-SCI-5892</u> _____ <u>May 10, 2011</u> Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

Commented [pn1]:

DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: Live animal work*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
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Ryan Clarke	USDA, APHIS, VS	Investigator
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Name	Address	Role in Study
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USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
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National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2017
Proposed Study Completion/Archive Date: October 1, 2018

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

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Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

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8. Methods/Procedures

A total of 46 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 10 seronegative and 36 seropositive - 2 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 4 seronegative bulls captured in late winter/spring 2011, 2012, and 2013 if needed as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute.

Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Commented [pn2]: Only semi-annually?

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of four pastures approximately 23 acres each. Each pasture will contain 16-18 seropositives and 4 seronegatives and 2 bulls. If not enough animals are collected by spring 2012 then two test pastures will be established in 2012 and two test pastures in 2013. Seropositive bison in two pastures will receive one injection of GonaCon™ vaccine (containing 3000µg in 2 ml adjuvant) intramuscularly in the right side of the neck. The site of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining two pastures will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from December until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

Commented [pn3]: Do we want to do this?

Commented [mm4]: If we do do this, is this where we set up cameras?

In addition, serology for each of the cows, bulls, and calves will be monitored twice a year. All bison will be tested by serology and culture in February and in summer following calving. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

Commented [pn5]: Do we want to do this more to monitor trends in serology (vax vs nonvax)

At the end of the study, all ~~adult~~ adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Commented [mm6]: seropositive?

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain

negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions).

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 46 females, 4 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be trapped by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol
Medetomidine
Azaperone

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered, ~~as animals will be chemically immobilized prior to euthanasia, or~~ 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: _____

Date of Consultation: _____

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be administered either by captive bolt, shot with a high powered rifle, or chemically immobilized and administered, as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Concur:
IACUC Chair _____ Date _____

From: [Stephens, Stephanie H - APHIS](#)
To: [Eisemann, John D - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: Draft Letter to EPA about Sand Dunes Study
Date: Wednesday, October 05, 2011 1:12:35 PM

Thanks, John. I just saw Pauline's comments. If any other comments can be sent by midday tomorrow (Thursday), there should be no problem getting the letter finalized and submitted to EPA on Friday.

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: Eisemann, John D - APHIS
Sent: Wednesday, October 05, 2011 12:27 PM
To: Stephens, Stephanie H - APHIS
Cc: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: Draft Letter to EPA about Sand Dunes Study

Jack, Pauline, Matt:

I have attached a draft of the letter to EPA. Please take a look at it and let us know if this is still the protocol you intend to follow. I took it straight from the summary Pauline sent me.

It would be great if we could get it sent to EPA on Friday. Getting their approval should be straight forward, but we still need to send it through the State of CO (which should also be straight forward, but will require some time).

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

From: [McCollum, Matthew P - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: RE: Draft of bison transfer agreement
Date: Tuesday, February 25, 2014 8:44:06 AM

Perfect. Jack is out of the office until Friday though. Should we hand the bag to Don?

Matt

From: Clarke, Patrick R. - APHIS
Sent: Monday, February 24, 2014 5:29 PM
To: McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Subject: RE: Draft of bison transfer agreement

Changed a few lines here and there to refer to "an affiliated facility at Ft Collins". And of course made sure Dr Jack Ryan was left holding the bag by making him the signatory.

This is the version I just sent to Dave Hallac.

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: McCollum, Matthew P - APHIS
Sent: Monday, February 24, 2014 3:48 PM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Subject: Draft of bison transfer agreement

Here is a quick draft. Not sure if we should just get them to Brogan's on this and worry about the rest later. I didn't go into very much detail... What do you think?

Matt McCollum

Wildlife Disease Biologist
USDA/APHIS/VS
Wildlife/Livestock Disease Investigations Team
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 Office
(b) (6) Mobile

From: [Clarke, Patrick R. - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: RE: Draft of bison transfer agreement
Date: Monday, February 24, 2014 5:29:08 PM
Attachments: [APHIS_BisonTransferAgreement_Feb2013MM-PRC.docx](#)

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INTERAGENCY AGREEMENT
between the
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
and the
NATIONAL PARK SERVICE

ARTICLE I. BACKGROUND AND OBJECTIVES

To evaluate the use of assisted reproduction techniques as means of genetic preservation for bison that are infected with *Brucella abortus*. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

ARTICLE II. STATEMENT OF WORK

A. During the period of performance, up to 15 live bison (12 adult cows and 3 adult bulls) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park. The animals will be held by the Animal and Plant Health Inspection Service in fenced quarantine pastures in Corwin Springs, Montana prior to being transferred for use in a reproductive study at an affiliated research location (National Wildlife Research Center in Ft Collins, CO). The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to evaluate embryos, offspring, and recipients for transmission of brucellosis via embryo transfer when in vivo and in vitro produced embryos are generated from cows and bulls with various titers of *Brucella abortus*. The rationale for this experiment is proof of principle that brucellosis-free embryos can be generated using oocytes and semen from infected animals without transmission of disease to embryo recipients or offspring.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

D. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

ARTICLE III. TERM OF AGREEMENT

The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

ARTICLE IV. KEY OFFICIALS

National Park Service
Yellowstone Center for Resources
Rick Wallen, Wildlife Biologist
P.O. Box 168
Yellowstone National Park, WY 82190
307-344-2285

Animal and Plant Health Inspection Service
Veterinary Services
Jack Rhyan, DVM
National Wildlife Research Center
Fort Collins, CO 80521
970-266-6140

ARTICLE V. PAYMENT

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status. Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure should be consigned to a quarantine location for further diagnostics, directly to a managed for public trust conservation program to supplement population genetic diversity, to an introduction program to establish a new conservation population of wild bison, or if no such opportunities exist, to a private not-for-profit bison conservation program. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis-free bison to any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

ARTICLE VII. PRIOR APPROVAL

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq.,

16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in a reproductive study at an affiliated research location (National Wildlife Research Center in Ft Collins, CO).

ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

ARTICLE IX. TERMINATION

Either party may terminate the agreement by providing 14 days advance written notice to the other party.

ARTICLE X. AUTHORIZING SIGNATURES

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: _____
Name: Daniel N. Wenk
Title: Superintendent, Yellowstone NP
Date: February ____, 2013

Signature: _____
Name: Jack Rhyan
Title: APHIS Veterinary Officer
Date: February ____, 2013

Signature: _____
Name: Tina Holland
Title: Contracting Officer
Date: February ____, 2013

Signature: _____
Name: _____
Title: _____
Date: February ____, 2013

From: [Rhyan, Jack C - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: Draft of bison transfer agreement
Date: Thursday, February 27, 2014 11:30:32 AM

I think you guys must've been at the top of the class in the "How to write Government Fluff" remedial training course! Wow! You're great! You're in the perfect job for your skill set! (Good job-shur nuff)

Jack

From: Clarke, Patrick R. - APHIS
Sent: Monday, February 24, 2014 5:29 PM
To: McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS
Cc: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS
Subject: RE: Draft of bison transfer agreement

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Matt McCollum

Wildlife Disease Biologist
USDA/APHIS/VS
Wildlife/Livestock Disease Investigations Team
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 Office
(b) (6) Mobile

From: [Jack C Rhyan](#)
To: [Rebecca K Frey](#)
Cc: [Brian J McCluskey](#); [Pauline Nol](#); [Matt McCollum](#)
Subject: Re: draft of contraception protocol
Date: Friday, February 11, 2011 4:12:00 PM
Attachments: [ImmunocontBisonProject_2-11-11.doc](#)

Becky,

Here it is with the latest changes. I want you and Brian to look it over one more time and then I think it'll be ready to share with YNP. We debated how much to play up the negatives going for conservation. I didn't make any big deal because we will need to outline exactly how that will be done and I'm not sure yet. Depends alot on Montana's legislative session.

Jack

(See attached file: [ImmunocontBisonProject_2-11-11.doc](#))

☐ Rebecca K Frey---02/10/2011 08:36:51 AM---Hi Jack, Is this finalized yet? Can we share with YNP?

**Rebecca K
Frey/MT/APHIS/USDA**

02/10/2011 08:32 AM

To: Jack C Rhyan/CO/APHIS/USDA@USDA
cc

Subject: Re: 'nuther draft of contraception protocol



Hi Jack,

Is this finalized yet? Can we share with YNP?

Becky Frey

☐ Jack C Rhyan---02/01/2011 02:57 PM MST---

From: Jack C Rhyan
To: Rebecca Frey; Matt McCollum; Pauline Nol
Cc:
Date: 02/01/2011 02:57 PM MST
Subject: 'nuther draft of contraception protocol

Please check it out and make any suggestions. this one has both seronegative calves and adults that pass quarantine being used for conservation. The devil is in the details so we will have to carefully design the process by which the critters are "used for conservation."

Jack

[attachment "ImmunocontBisonProject_2-1-11.doc" deleted by Rebecca K Frey/MT/APHIS/USDA]

Proposed Project:**DRAFT**

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaConTM vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December until July. During the three

abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.

2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

From: [Clarke, Patrick R. - APHIS](#)
To: [McCluskey, Brian J - APHIS](#)
Cc: [Herriott, Donald E - APHIS](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: Draft Version of Bison Quarantine Protocol
Date: Wednesday, January 04, 2012 2:49:22 PM

Brian,

We just received the final test results on the 2nd cohort yesterday. We haven't started any formal analysis as yet.....we were under the impression you needed this sooner rather than later for the IBMP folks.....that even though the study was incomplete, that we could/should produce a best "guess" protocol based on our experience and the culture/test results in hand.

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: McCluskey, Brian J - APHIS
Sent: Wednesday, January 04, 2012 12:36 PM
To: Clarke, Patrick R. - APHIS
Cc: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: RE: Draft Version of Bison Quarantine Protocol

Ryan,

I wouldn't mind seeing the hard data as well. What kind of analysis was done to determine the overall time periods of quarantine for the various classes of bison? The study seems to lend itself well to a survival analysis.

Brian

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 04, 2012 11:41 AM
To: McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Subject: Draft Version of Bison Quarantine Protocol

Brian, Don

The Bison Group (Jack, Matt, Pauline, Becky and I) put our heads together and came up with this draft. Even though the data from the BQFS has not been fully evaluated, this document is our recommendation for a quarantine protocol to be used for an Approved Bison Quarantine Facility. This protocol is based on our experience from the BQFS.

The original protocol (2003 UM & R), as put together by a variety of experts based primarily on their prior experience and knowledge of the disease in cattle, was essentially untried. We felt that the BQFS validated and proved this original protocol for bison, which is why what we have sent you does not radically depart from the original framework. The one significant change was removing the stipulation for post quarantine testing.

We passed this by John B., Arnie G., Mark C. and Don Evans. There were no major concerns except that Don actually wanted to see the data to get a feel for the numbers of animals/tests used in the study.

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#)
Subject: RE: Final GonaCon Study
Date: Friday, December 09, 2011 3:13:00 PM
Attachments: [ACUCBisonGonaConStudyfinal \(2\).pdf](#)
[ACUC Proposal GonaConBisonStudy2011final5.23.11.docx](#)
[ACUC Proposal GonaConBisonStudy2011amendmentform7.1.11.docx](#)

Hi Ryan,

I've attached the ACUC protocol and the ACUC amendment. In my files, the final version of the protocol is in the form of the ACUC documents. All my versions of the protocol we picked apart are still all picked apart☺ I hope this helps. I still need to push the NWRC protocol through and will give you that when it's done.

Have a great weekend and see you next week!

Pauline

From: Clarke, Patrick R. - APHIS
Sent: Friday, December 09, 2011 12:05 PM
To: Nol, Pauline - APHIS
Subject: Final GonaCon Study

Pauline ,

Could you send me the final version of the GonaCon Bison Study proposal? I have a couple versions but I think they were written before we had our final discussions with NPS.

Thanks,

P. Ryan Clarke, DVM

Regional Epidemiologist-GYA

USDA/APHIS/VS/WR

Belgrade, Montana

406-388-5162

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____</p> <p style="text-align: center;">Permit(s) description Number Date</p>

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☒ *Data analysis, interpretation and reporting*
 - ☒ *Other: _____ Live animal work _____*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

on the occurrence of pregnancy and abortion or calving of infected animals. GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

SIGNATURE PAGE

Study Director

Jade C. Ryan

Date

5/16/2011

Concur

IACUC Chair

Date

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <p>_____</p> <p style="text-align: left;">Permit(s) description Number Date</p>

DESCRIPTION OF ACTIVITIES

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- ☐ *Advisory Committee participation*
 - ☒ *Manuscript/review article collaboration*
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	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

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Other Investigators, Collaborators, Cooperators, and Consultants		
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Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

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(Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

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7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute.

Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain

negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
 Xylazine- 0.07 mg/kg, IM dart

 Carfentanil-0.005-0.01 mg/kg, IM dart
 Xylazine- 0.07 mg/kg, IM dart

 Butorphenol- 0.03-0.06 mg/kg, IM dart
 Medetomidine- 0.01-0.02 mg/kg
 Azaperone- 0.02 mg/kg

Reversal for narcotics:

 Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
 Tolazoline-300 mg as needed IM

Reversal for BAM:

 Atipamezole 0.0375-0.03 mg/kg IM
 Naltrexone 0.05-0.125mg/kg IM
 Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyon, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

SIGNATURE PAGE

Study Director _____ Date_____

Concur

IACUC Chair _____ Date_____

**Amendment Form
Animal Care and Use Protocol
Bison Quarantine Facility Institutional Animal Care and Use Committee**

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

Amendments:

DESCRIPTION OF ACTIVITIES

The end date to this project should be changed to October 1, 2019

STUDY PROTOCOL

2. Testing Facilities

Montana Veterinary Diagnostic Laboratory will also be receiving serum for Brucellosis testing.

7. Objective/Hypotheses

In this section, Major Objective (2) will be added and will deal with evaluating efficacy of GonaCon™. Consequently, an additional hypothesis (2) will be added. The original Major Objective number 3 will be changed to come under the Minor Objectives section.

This section will read as follows:

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive in female *B. abortus*-infected bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *B. abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancy rates in female *B. abortus*-infected bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

8. Methods/Procedures

Serologic testing for anti-GnRH antibodies will also be conducted in this project. The paragraph below will be added to the section.

Serology evaluating antibody production against GnRH will be conducted at the National Wildlife Research Center. Serology will be conducted prior to vaccination and at least annually thereafter.

10. Experimental Design and Statistical Analyses

This section will be changed to add sample size justification in reference to efficacy testing of GonaCon™ to prevent pregnancies in female bison. In addition, we will add the term “shedding” as a response variable in addition to “abortion”. This section will read as follows:

If we expect an abortion/shedding rate of 5-10% in the vaccinated group and a 30% abortion/shedding rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions/shedding occurrence). Two replicates of the two pastures will be conducted.

As we consider power to be acceptable at a level of approximately 80% for evaluating vaccine efficacy, the number of animals involved in this study is appropriate. The vaccine will be deemed successful if the number of births in non-vaccinates exceeds that of vaccinates by 60% or more. Using a power calculation in SAS (power for comparing 2 independent proportions), a sample size of 10 or greater per group was calculated to be sufficient in order to determine efficacy of the vaccine under the above-stated power constraint.

SIGNATURE PAGE

Study Director _____ Date_____

Concur

IACUC Chair _____ Date_____

From: [McCollum, Matthew P - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: Final Report on 13-021992 For DR Jack C Rhyan
Date: Friday, March 21, 2014 9:41:18 AM

Yes

Sent from my iPhone

> On Mar 20, 2014, at 2:23 PM, "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov> wrote:

>

> These are the bulls we shipped you that you cultured from the semen?

>

> Becky

> USDA APHIS VS

> Sent from my iPhone

>

>> On Mar 20, 2014, at 1:54 PM, "McCollum, Matthew P - APHIS" <Matt.McCollum@aphis.usda.gov> wrote:

>>

>> Report from the bulls that were shedders. Interestingly, no hits on culture...

>>

>> Matt

>>

>> -----Original Message-----

>> From: Rhyan, Jack C - APHIS

>> Sent: Thursday, March 20, 2014 9:34 AM

>> To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

>> Subject: FW: Final Report on 13-021992 For DR Jack C Rhyan

>>

>>

>>

>> -----Original Message-----

>> From: APHIS-NVSL Case Coordinator - APHIS

>> Sent: Thursday, March 20, 2014 7:25 AM

>> To: Rhyan, Jack C - APHIS

>> Subject: Final Report on 13-021992 For DR Jack C Rhyan

>>

>> Final Report on 13-021992 For DR Jack C Rhyan

>> <13-021992_1.pdf>

From: [Nelson, Janell - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Cc: [Eisemann, John D - APHIS](#)
Subject: RE: FOIA Search - 12-00575
Date: Wednesday, April 11, 2012 11:30:52 AM

VS did already respond to this request. The summary below is not completely accurate, but anyway.

From: Nol, Pauline - APHIS
Sent: Wednesday, April 11, 2012 11:27 AM
To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Cc: Eisemann, John D - APHIS
Subject: FW: FOIA Search - 12-00575

FYI-It looks like this recently got sent only to WS (see below) but the date is January. Wasn't this already fulfilled?

Pauline

From: Eisemann, John D - APHIS
Sent: Wednesday, April 11, 2012 11:22 AM
To: Nol, Pauline - APHIS
Subject: FW: FOIA Search - 12-00575

FYI

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

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From: Romines, Janean - APHIS
Sent: Wednesday, April 11, 2012 8:06 AM

To: Eisemann, John D - APHIS

Cc: Clark, Larry - APHIS; Tobin, Mark E - APHIS; Deliberto, Thomas J - APHIS; Freeman, Nancy - APHIS

Subject: FW: FOIA Search - 12-00575

Hi John-

Please see the email below regarding responsive records to the attached request. WS did not originally receive this request; it went to VS and they provided a negative response. VS is saying this was a WS study. Can you please check to see if you have records that would be responsive?

Also, I will be on a conference call tomorrow with APHIS FOIA and OGC regarding the release of the GonaCon formulation. I have requested that the information be withheld under FIFRA. EPA does use the FIFRA Statute to withhold confidential information and in a 2010 FOIA request we used this same statute to withhold the GonaCon information; however, there seems to be a question on whether or not we can protect this because we are not a private entity. The government does not get many privacy rights. I will keep you posted.

Thanks for doing a search for the records and returning the records (if found) or a negative reply. Please call if you have questions.

Janean Romines

Staff Officer/Wildlife Biologist
Operational Support Staff
USDA/APHIS/Wildlife Services
4700 River Road
Unit 87, Rm. 2B-08.2
Riverdale, MD 20737
Off: 301.851.3996
Cell: (b) (6)
Fax: 301.734.5157

From: Camp, Celeste - APHIS

Sent: Wednesday, April 11, 2012 7:04 AM

To: Doerrer, Michael R - APHIS; Hamm, Shannon R - APHIS; Romines, Janean - APHIS

Cc: Bundy, Mildred O - APHIS; Tuszynski, Carol A - APHIS; Ragin, Cindy N - APHIS

Subject: RE: FOIA Search - 12-00575

Janean, would you please take a look at the attached request and let me know if WS would have the requested information? As you can see from VS' response, they do not have any responsive records, but has indicated this was WS' study.

Celeste

From: Doerr, Michael R - APHIS
Sent: Tuesday, April 10, 2012 5:55 PM
To: Hamm, Shannon R - APHIS
Cc: Bundy, Mildred O - APHIS; Camp, Celeste - APHIS; Tuszyński, Carol A - APHIS; Ragin, Cindy N - APHIS
Subject: Re: FOIA Search - 12-00575

It is a Wildlife Services study.

Michael Doerr

Chief Operating Officer

USDA-APHIS-Veterinary Services

On Apr 10, 2012, at 5:49 PM, "Hamm, Shannon R - APHIS"
<Shannon.R.Hamm@aphis.usda.gov> wrote:

<image001.jpg>

Did you not conduct a cooperative agreement or contract for the study?

From: Bundy, Mildred O - APHIS
Sent: Tuesday, April 10, 2012 2:17 PM
To: Hamm, Shannon R - APHIS; Camp, Celeste - APHIS
Cc: Doerr, Michael R - APHIS; Tuszyński, Carol A - APHIS; Ragin, Cindy N - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search - 12-00575

Pursuant to appropriation questions for the Bison Quarantine Feasibility Study, there wasn't an earmark for the Bison Quarantine Feasibility Study, that project is funded as part of the brucellosis program. There is no separate budget for that project, therefore there is no way to identify a "balance of funds" for the project and we did not separately track spending on that project.

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Frey, Rebecca K \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: RE: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011
Date: Friday, June 10, 2011 8:24:14 AM

Nope,

I've not seen anything pertaining to a budget.

Matt

From: Frey, Rebecca K (APHIS)
Sent: Friday, June 10, 2011 8:15 AM
To: Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: Fw: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Hi,
I think our project proposal will satisfy this, though I was wondering if you had anything on budget for this. I have never seen anything, other than knowing what the leases are.
Thanks,
Becky
Becky Frey

From: Janell R Nelson
Sent: 06/09/2011 10:58 PM GMT
To: Patrick Clarke; Jack Rhyan; Rebecca Frey
Cc: Janell Nelson
Subject: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Drs. Clarke, Rhyan & Frey:

We have received the FOIA search request attached below.
As you are aware, we now have five (5) work days to:
-search for the appropriate records (paper and electronic),
-create copies of the records, and
-deliver them and the completed Request for Document Search form to the WRO (to my attention).

The FOIA office will redact any Privacy Act-protected information from the records we provide to the FOIA Liaison. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise me by e-mail when the response documents are en route to this office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O (APHIS)
Sent: Thursday, June 09, 2011 12:39 PM
To: Nelson, Janell (APHIS)
Subject: New Request: FOIA 11-564

Hi Janelle: Hope all is well.

From: [Nol, Pauline \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#)
Subject: Re: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011
Date: Friday, June 10, 2011 10:04:14 AM

I concur. No budgets have been produced as far as I am aware.

P

From: McCollum, Matthew P (APHIS)
Sent: Friday, June 10, 2011 09:24 AM
To: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS)
Subject: RE: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Nope,

I've not seen anything pertaining to a budget.

Matt

From: Frey, Rebecca K (APHIS)
Sent: Friday, June 10, 2011 8:15 AM
To: Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: Fw: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Hi,
I think our project proposal will satisfy this, though I was wondering if you had anything on budget for this. I have never seen anything, other than knowing what the leases are.
Thanks,
Becky
Becky Frey

From: Janell R Nelson
Sent: 06/09/2011 10:58 PM GMT
To: Patrick Clarke; Jack Rhyan; Rebecca Frey
Cc: Janell Nelson
Subject: FOIA Search Request -- FOIA 11-564 DUE 6/13/2011

Drs. Clarke, Rhyan & Frey:

We have received the FOIA search request attached below.
As you are aware, we now have five (5) work days to:
-search for the appropriate records (paper and electronic),
-create copies of the records, and
-deliver them and the completed Request for Document Search form to the WRO (to my attention).

The FOIA office will redact any Privacy Act-protected information from the records we provide to the FOIA Liaison. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise me by e-mail when the response documents are en route to this office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O (APHIS)
Sent: Thursday, June 09, 2011 12:39 PM
To: Nelson, Janell (APHIS)
Subject: New Request: FOIA 11-564

Hi Janelle: Hope all is well.

From: [Bundy, Mildred O - APHIS](#)
To: [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Tuesday, February 21, 2012 6:00:06 AM
Attachments: [image001.png](#)

This was what was sent to Janell and I said that this information would fulfill the request, but I never received this information. This is reading: that NVSL does produce the reports and you guys get the reports. If so, you would have the reports right and then you would send them to me? I am confused.

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

From: Bundy, Mildred O - APHIS
Sent: Tuesday, February 21, 2012 7:29 AM
To: Nelson, Janell - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

FYI: I never received anything from NVSL.

From: Nelson, Janell - APHIS
Sent: Thursday, January 26, 2012 3:39 PM
To: Bundy, Mildred O - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Mildred:

By my calendar, it is one week overdue; but we believed that your e-mail from 1/12/2012 stated the information from NVSL was sufficient to answer the request. Please clarify. We are not trying to withhold anything, we just thought it had been taken care of by the folks with the original documents.

Janell

Yes, it will fulfill the request. Thanks

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyon, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Bundy, Mildred O - APHIS
Sent: Thursday, January 26, 2012 1:28 PM
To: Clarke, Patrick R. - APHIS; Nelson, Janell - APHIS; Rhyon, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

THIS CASE IS ALMOST 2 WEEKS OVERDUE!!!!!!!!!! Can someone please forward as soon as possible.
Thanks

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyon, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyan:

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Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS

Cc: Bundy, Mildred O - APHIS

Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR
REQUEST #: 2012-APHIS-01161F

REQUESTER: GEIST
DUE TO FOIA: 1/18/12

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SEARCH START DATE:

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From: [Bundy, Mildred O - APHIS](#)
To: [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Rhyen, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Tuesday, February 21, 2012 5:28:34 AM
Attachments: [image001.png](#)

FYI: I never received anything from NVSL.

From: Nelson, Janell - APHIS
Sent: Thursday, January 26, 2012 3:39 PM
To: Bundy, Mildred O - APHIS; Clarke, Patrick R. - APHIS; Rhyen, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
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Mildred:

By my calendar, it is one week overdue; but we believed that your e-mail from 1/12/2012 stated the information from NVSL was sufficient to answer the request. Please clarify. We are not trying to withhold anything, we just thought it had been taken care of by the folks with the original documents.

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Yes, it will fulfill the request. Thanks

From: Clarke, Patrick R. - APHIS
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Staff Assistant, VS Western Region
970-494-7400

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Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO:	WR	REQUESTER: GEIST
REQUEST #:	2012-APHIS-01161F	DUE TO FOIA: 1/18/12

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Name

Title

Office and Phone

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To: [Clarke, Patrick R. - APHIS](#); [Nelson, Janell - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Tuesday, February 21, 2012 5:25:45 AM
Attachments: [image001.png](#)

Can someone please give me this overdue information? I am amazed that after several requests to have this information submitted to me, that no one has provided me the documents. The FOIA Office is upset that the Western Region is not in compliance.

If you do not have the information, could I please have the courtesy of someone giving me an update?

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS
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Regional Epidemiologist-GYA
Belgrade, MT
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Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS

Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyan:

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Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO:	WR	REQUESTER: GEIST
REQUEST #:	2012-APHIS-01161F	DUE TO FOIA: 1/18/12

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Search conducted by:

Name

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Office and Phone

Missing Document Explanation/Special Notes:

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To: [Nelson, Janell - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012 - Need INFORMATION ASAP - thanks
Date: Monday, January 30, 2012 8:38:59 AM
Attachments: [image001.png](#)

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 7:00 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
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Sent: Tuesday, January 10, 2012 7:17 AM
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Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR
REQUEST #: 2012-APHIS-01161F

REQUESTER: GEIST
DUE TO FOIA: 1/18/12

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Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Thursday, January 26, 2012 1:38:40 PM
Attachments: [image001.png](#)

Mildred:

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APHIS

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Search time* (professional): _____
 *Does not include photocopying time: _____
Review time (professional): _____
Search conducted by: _____

Name	Title	Office and Phone
------	-------	------------------

Missing Document Explanation/Special Notes:

From: [Bundy, Mildred O - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Nelson, Janell - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Thursday, January 19, 2012 11:32:00 AM
Attachments: [image001.png](#)

STATUS PLEASE.....

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyan:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

- search** for the appropriate records (paper and electronic),
- create copies** of the records, and
- deliver them** and the completed Request for Document Search form to the FOIA liaison

(**Mildred Bundy** -- her address is listed below).

Do not create new documents (e.g. lists, tables, any kind of compilation from records) in response to FOIA requests. The FOIA office will redact any Privacy Act-protected information from the records you provide to Ms. Bundy. We may not withhold records from the FOIA office; if you believe that certain information on the records is protected by the Privacy Act, you are encouraged to note that fact on the Request for Document Search cover sheet. Additionally, we may NOT release records directly to the requestor. Only the FOIA office may release information to the requestor.

Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR **REQUESTER:** GEIST
REQUEST #: 2012-APHIS-01161F **DUE TO FOIA:** 1/18/12

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: mildred.bundy@aphis.usda.gov, if sending by mail, send to USDA, APHIS, **MILDRED BUNDY**, 4700 Riverdale Road, Riverdale, MD 20737.

SEARCH START DATE:

Search time* (clerical): _____

Search time* (professional): _____

***Does not include photocopying time:** _____

Review time (professional): _____

Search conducted by:

Name

Title

Office and Phone

Missing Document Explanation/Special Notes:

From: [Bundy, Mildred O - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Nelson, Janell - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Thursday, January 12, 2012 12:38:54 PM
Attachments: [image001.png](#)

Yes, it will fulfill the request. Thanks

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyan:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

- search** for the appropriate records (paper and electronic),
- create copies** of the records, and
- deliver them** and the completed Request for Document Search form to the FOIA liaison (**Mildred Bundy** -- her address is listed below).

Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

SEARCH START DATE:
Search time* (clerical): _____
Search time* (professional): _____
 *Does not include photocopying time: _____
Review time (professional): _____
Search conducted by: _____

Missing Document Explanation/Special Notes:

From: [Clarke, Patrick R. - APHIS](#)
To: [Nelson, Janell - APHIS](#); [Rhyon, Jack C - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Bundy, Mildred O - APHIS](#); [Linfield, Thomas F - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Wednesday, January 11, 2012 8:26:55 AM
Attachments: [image001.png](#)

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyon, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyon:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

- search** for the appropriate records (paper and electronic),
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Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR **REQUESTER:** GEIST
REQUEST #: 2012-APHIS-01161F **DUE TO FOIA:** 1/18/12

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: mildred.bundy@aphis.usda.gov, if sending by mail, send to USDA, APHIS, MILDRED BUNDY, 4700 Riverdale Road, Riverdale, MD 20737.

SEARCH START DATE:

Search time* (clerical): _____

Search time* (professional): _____

***Does not include photocopying time:** _____

Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
------	-------	------------------

Missing Document Explanation/Special Notes:

From: [Rhyan, Jack C - APHIS](#)
To: [Quance, Christine R - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Linfield, Thomas F - APHIS](#); [Bundy, Mildred O - APHIS](#); [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012
Date: Tuesday, February 21, 2012 10:52:44 AM
Attachments: [image001.png](#)

Chris,

We've all been talking about you but I thought maybe we should talk with you. Hmmm. As you can see, there was a FOIA request for which we think you have the needed info.

We assumed the FOIA folks were talking with you but apparently not. So now, just before they haul us off to federal prison for being so tardy, I thought maybe you should be given the opportunity to save us all.

Have you heard of this? Can you provide this info?

Is there somewhere else to get it?

I'll send you the original request if I still have it.

Jack

From: Bundy, Mildred O - APHIS
Sent: Tuesday, February 21, 2012 5:29 AM
To: Nelson, Janell - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

FYI: I never received anything from NVSL.

From: Nelson, Janell - APHIS
Sent: Thursday, January 26, 2012 3:39 PM
To: Bundy, Mildred O - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Mildred:

By my calendar, it is one week overdue; but we believed that your e-mail from 1/12/2012 stated the information from NVSL was sufficient to answer the request. Please clarify. We are not trying to withhold anything, we just thought it had been taken care of by the folks with the original documents.

Janell

Yes, it will fulfill the request. Thanks

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, January 11, 2012 10:27 AM
To: Nelson, Janell - APHIS; Rhyan, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E -

APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: Bundy, Mildred O - APHIS

Sent: Thursday, January 26, 2012 1:28 PM

To: Clarke, Patrick R. - APHIS; Nelson, Janell - APHIS; Rhyon, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

THIS CASE IS ALMOST 2 WEEKS OVERDUE!!!!!!!!!! Can someone please forward as soon as possible.
Thanks

From: Clarke, Patrick R. - APHIS

Sent: Wednesday, January 11, 2012 10:27 AM

To: Nelson, Janell - APHIS; Rhyon, Jack C - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS

Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS; Linfield, Thomas F - APHIS

Subject: RE: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Janell,

We heard this was coming and gave James Higgins and Chris Quance at NVSL a heads up. They produce the genotyping reports and send them to us electronically. If they supplied what they produced in 2010 and 2011 for MT, ID, and WY, would this not fulfill the FOIA request?

Ryan

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Tuesday, January 10, 2012 5:00 PM
To: Rhyon, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01161-F DUE 1/18/2012

Drs. Herriott, McCluskey, Clarke, & Rhyon:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

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- create copies** of the records, and
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Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Tuesday, January 10, 2012 7:17 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: New FOIA Search Memo - 2012-APHIS-01161-F

TO: WR
REQUEST #: 2012-APHIS-01161F

REQUESTER: GEIST
DUE TO FOIA: 1/18/12

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SEARCH START DATE:

Search time* (clerical): _____

Search time* (professional): _____

***Does not include photocopying time:** _____

Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
------	-------	------------------

Missing Document Explanation/Special Notes:

From: [Rhyan, Jack C - APHIS](#)
To: [Nelson, Janell - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Hepburn, Tania S - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Bartling, David L - APHIS](#); [Bundy, Mildred O - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01470-F DUE-- 2/15/2012
Date: Wednesday, February 08, 2012 12:22:05 PM
Attachments: [image001.png](#)

I don't think I have any of this. The pilot business probably belongs to WS. Please let me know if you need me to dig around.

Thanks,
Jack

From: Nelson, Janell - APHIS
Sent: Wednesday, February 08, 2012 9:47 AM
To: McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Hepburn, Tania S - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bartling, David L - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01470-F DUE-- 2/15/2012

Drs. McCluskey, Herriott, Clarke, & Rhyan, and Tania:

We have received the attached FOIA search request.
As you are aware, we now have five (5) work days to:
-**search** for the appropriate records (paper and electronic),
-**create copies** of the records, and
-**deliver them** and the completed Request for Document Search form to the FOIA liaison (**Mildred Bundy** -- USDA, APHIS, MILDRED BUNDY, 4700 Riverdale Road, Room 4B02.9 Riverdale, MD 20737).

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Please advise the FOIA liaison by e-mail (and cc: me) when the response documents are en route to her office.

Janell Nelson
Staff Assistant, VS Western Region
970-494-7400

From: Bundy, Mildred O - APHIS
Sent: Wednesday, February 08, 2012 6:53 AM

To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: NEW FOIA REQUEST: Case #: 2012-APHIS-01470-F

TO: WR **REQUESTER:** GEIST
REQUEST #: 2012-APHIS-01470F **DUE TO FOIA:** 2/15/2012

Attached is a FOIA request for documents maintained by your office. You must search in every place where a reasonably knowledgeable professional could expect to find responsive records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

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SEARCH START DATE: _____
Search time* (clerical): _____
Search time* (professional): _____
*Does not include photocopying time
Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
Missing Document Explanation/Special Notes:		

From: [Frey, Rebecca K - APHIS](#)
To: [Nelson, Janell - APHIS](#); [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [Hepburn, Tania S - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Bartling, David L - APHIS](#); [Bundy, Mildred O - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01470-F DUE-- 2/15/2012
Date: Wednesday, February 08, 2012 2:04:01 PM
Attachments: [image001.png](#)

I don't have any of this information nor would I have been on any correspondence. To my knowledge, # 4 does not exist. I agree with Jack that WS would have more information on the aircraft.

Becky

Rebecca Frey
Wildlife Biologist/Disease Specialist
Greater Yellowstone Area
406-333-4425

From: Nelson, Janell - APHIS
Sent: Wednesday, February 08, 2012 9:47 AM
To: McCluskey, Brian J - APHIS; Herriott, Donald E - APHIS; Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Hepburn, Tania S - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Bartling, David L - APHIS; Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01470-F DUE-- 2/15/2012

Drs. McCluskey, Herriott, Clarke, & Rhyan, and Tania:

We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:

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Janell Nelson
Staff Assistant, VS Western Region

From: Bundy, Mildred O - APHIS
Sent: Wednesday, February 08, 2012 6:53 AM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: NEW FOIA REQUEST: Case #: 2012-APHIS-01470-F

TO: **WR** **REQUESTER:** GEIST
REQUEST #: 2012-APHIS-01470F **DUE TO FOIA:** 2/15/2012

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SEARCH START DATE: _____
Search time* (clerical): _____
Search time* (professional): _____
*Does not include photocopying time
Review time (professional): _____

Search conducted by:

Name	Title	Office and Phone
Missing Document Explanation/Special Notes:		

From: [Frey, Rebecca K - APHIS](#)
To: [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Bundy, Mildred O - APHIS](#)
Cc: [Herriott, Donald E - APHIS](#); [McCluskey, Brian J - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Re: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012
Date: Thursday, March 22, 2012 11:32:55 AM

<http://www.buffalofieldcampaign.org/media/press1112/pressreleases1112/031312.html>

From: Nelson, Janell - APHIS
Sent: Thursday, March 22, 2012 12:30 PM
To: Clarke, Patrick R. - APHIS; Bundy, Mildred O - APHIS
Cc: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012

I'm not sure I understand where your question is coming from unless it's because all of BFC's recent requests are initiated by a lawyer.

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Janell R. Nelson

Staff Assistant

USDA APHIS VS Western Region | 2150 Centre Ave., Bldg B MS3E13 | Fort Collins, Colorado 80526 | 970-494-7400 | janell.r.nelson@aphis.usda.gov

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 22, 2012 11:03 AM
To: Nelson, Janell - APHIS; Bundy, Mildred O - APHIS
Cc: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Subject: RE: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012

Janell and Mildred,

Are we being sued over the previous FOIA requests? If so, are we supposed to communicate through attorneys now? Should the records we gather for this FOIA request go to our lawyers first?

P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Thursday, March 22, 2012 9:46 AM
To: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. -

APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012

Drs. Herriott, McCluskey, Rhyan, Clarke, Nol, Frey & McCollum:
We have received the attached FOIA search request.

As you are aware, we now have five (5) work days to:
-search for the appropriate records (paper and electronic),
-create copies of the records, and
-deliver them and the completed Request for Document Search form to the FOIA liaison
(**Mildred Bundy** -- if sending by mail, send to USDA, APHIS, MILDRED BUNDY, 4700 Riverdale
Road, Riverdale, MD 20737).

Do not create new documents (e.g. lists, tables, any kind of compilation from records) in
response to FOIA requests. The FOIA office will redact any Privacy Act-protected
information from the records you provide to Ms. Bundy. We may not withhold records from
the FOIA office; if you believe that certain information on the records is protected by the
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office may release information to the requestor.

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route to her office.

Janell R. Nelson
Staff Assistant

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970-494-7400 | janell.r.nelson@aphis.usda.gov

From: Bundy, Mildred O - APHIS
Sent: Wednesday, March 21, 2012 6:42 PM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: NEW SEARCH Search Memo - Case #: 2012-APHIS-01942-F

NOTE: This was also sent to WS.

TO: VS-WR **REQUESTER:** Seay

REQUEST #: FOIA-12-01911 **DUE TO FOIA:** 03/29/2012

Attached is a FOIA request for documents maintained by your office. You must search in
every place where a reasonably knowledgeable professional could expect to find responsive

records. The search obligation goes far beyond the file cabinet or file folders. It includes searches of electronic media, such as computer hard drives, e-mail, electronic calendars, archives, servers, cd's, thumb drives etc.

Please complete this page and return it with the responsive records. If providing records electronically, please e-mail them to: mildred.bundy@aphis.usda.gov, if sending by mail, send to Mildred Bundy, USDA, APHIS, 4700 Riverdale Road, 4B02.25, Riverdale, MD 20737.

SEARCH START DATE: _____

Search time* (clerical): _____

Search time* (professional): _____

*Does not include photocopying time

Review time (professional): _____

Search conducted by:

Name

Title

Office and Phone

Missing Document Explanation/Special Notes:

*****PLEASE NOTE: Agency records retention periods are affected by this FOIA/PA request. DO NOT DESTROY ORIGINALS for a minimum of 3 years. Please see APHIS Records Management Handbook: Info 8 - Privacy Act Requests and Info 9 - FOIA Requests.**

From: [Bundy, Mildred O - APHIS](#)
To: [Nelson, Janell - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Cc: [Herriott, Donald E - APHIS](#); [McCluskey, Brian J - APHIS](#); [Rhyen, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Re: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012
Date: Thursday, March 22, 2012 11:34:22 AM

Janelle you are correct. If we have been tasked to retrieve this information through a viable FOIA request - we must provide requested documents. FOIA is aware of any litigation.

From: Nelson, Janell - APHIS
Sent: Thursday, March 22, 2012 12:30 PM
To: Clarke, Patrick R. - APHIS; Bundy, Mildred O - APHIS
Cc: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyen, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
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Staff Assistant

USDA APHIS VS Western Region | 2150 Centre Ave., Bldg B MS3E13 | Fort Collins, Colorado 80526 | 970-494-7400 | janell.r.nelson@aphis.usda.gov

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To: Nelson, Janell - APHIS; Bundy, Mildred O - APHIS
Cc: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyen, Jack C - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
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P. Ryan Clarke
USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

From: Nelson, Janell - APHIS
Sent: Thursday, March 22, 2012 9:46 AM
To: Herriott, Donald E - APHIS; McCluskey, Brian J - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; McCollum, Matthew P - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012

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From: Bundy, Mildred O - APHIS
Sent: Wednesday, March 21, 2012 6:42 PM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: NEW SEARCH Search Memo - Case #: 2012-APHIS-01942-F

NOTE: This was also sent to WS.

TO:	VS-WR	REQUESTER: <u>Seay</u>
REQUEST #:	FOIA-12-01911	DUE TO FOIA: <u>03/29/2012</u>

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From: [Nelson, Janell - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Bundy, Mildred O - APHIS](#)
Cc: [Herriott, Donald E - APHIS](#); [McCluskey, Brian J - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: FOIA Search Request: 2012-APHIS-01942-F DUE 3/29/2012
Date: Thursday, March 22, 2012 11:30:08 AM

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USDA, APHIS, VS,WR
Regional Epidemiologist-GYA
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From: Bundy, Mildred O - APHIS
Sent: Wednesday, March 21, 2012 6:42 PM
To: Nelson, Janell - APHIS
Cc: Bundy, Mildred O - APHIS
Subject: NEW SEARCH Search Memo - Case #: 2012-APHIS-01942-F

NOTE: This was also sent to WS.

TO: VS-WR **REQUESTER:** Seay

REQUEST #: FOIA-12-01911 **DUE TO FOIA:** 03/29/2012

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*Does not include photocopying time

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Search conducted by:

Name

Title

Office and Phone

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From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: FREEDOM OF INFORMATION ACT REQUEST
Date: Thursday, October 20, 2011 11:25:30 AM

Looks like they have all of the information already. What else do they think we have?

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS VS WR
406-333-4425

From: Clarke, Patrick R. - APHIS
Sent: Thursday, October 20, 2011 11:15 AM
To: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: FW: FREEDOM OF INFORMATION ACT REQUEST

Looks like a FOIA request is in the pipeline for the BQFS.

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

From: Darrell Geist [<mailto:z@wildrockies.org>]
Sent: Thursday, October 20, 2011 11:00 AM
To: FOIA Officer
Cc: z@wildrockies.org; Clarke, Patrick R. - APHIS
Subject: FREEDOM OF INFORMATION ACT REQUEST

BUFFALO FIELD CAMPAIGN
P.O. BOX 957
WEST YELLOWSTONE, MONTANA 59758
(406) 646-0070 PHONE (406) 646-0071 FAX
<http://www.buffalofieldcampaign.org>
buffalo@wildrockies.org

October 20, 2011

Tonya Woods, FOIA/PA Officer
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 50
Riverdale, MD 20737-1232
Tel. 301-734-5267
Fax 301-734-5941
Email: FOIA.Officer@aphis.usda.gov

RE: FEDERAL FREEDOM OF INFORMATION ACT REQUEST

Ms. Woods:
Pursuant to the federal Freedom of Information Act (5 U.S.C. 552 et. seq.), Buffalo Field Campaign is filing this request for information.

Buffalo Field Campaign is a 501(c) (3) non-profit, public interest, grassroots media-based organization, which provides news reports directly to thousands of supporters which include concerned American citizens, and

tourists from around the globe, as well as to regional, national and international media.

We would prefer an electronic copy of this information but we would be happy to get a paper copy of anything that is not available electronically. We request the following documentation from USDA APHIS:

1. Records that disclose the status of bison captured inside Yellowstone National Park for consignment to USDA APHIS' quarantine feasibility study, specifically those records that account for births and deaths and causes for each death recorded of bison in quarantine.

According to publicly available records provided by USDA APHIS and Montana Fish, Wildlife & Parks:

1st cohort captured inside Yellowstone National Park and trucked to Corwin Springs quarantine in April 2005 with additional buffalo captured for quarantine in 2006.

- + 17 calves trucked in.
- + 85 calves trucked in.
- 3 buffalo sero-converted and were euthanized.
- 4 buffalo sero-converted and were euthanized.
- 2 of 3 "suspect" buffalo were euthanized.
- + 21 calves born in captivity 2008.
- 5 calves died at birth: 4 stillborn from dystocia, 1 rejected by mother.
- 2 mothers died after giving birth.
- 2 calves euthanized after mothers died.
- + 30 calves born in captivity 2009.
- 48 randomly selected buffalo were slaughtered "to determine latent infection. All were culture negative."

1st cohort of 87 buffalo - 33 adult females, 8 adult bulls, 16 yearlings, and 30 calves - trucked to Turner Enterprise Inc.'s Green Ranch in Montana February 17-18, 2010.

- + 21 calves born in captivity 2010.
- 4 buffalo died total: 1 orphaned calf died shortly after transport; yearling died from meningoencephalitis; 5 year old broke a leg; newborn calf died umbilical cord infection; 2 year old female struck by lightning.
- + 40 calves born in captivity 2011.

Buffalo in quarantine on Turner's Green Ranch: 143.

2nd cohort of 112 buffalo captured inside Yellowstone National Park for consignment to quarantine winter 2008.

- + 112 calves trucked in.
- 27 buffalo sero-converted and were euthanized.
- 4 buffalo died; 2 from "unknown causes" and 2 from "trauma related to handling."
- 41 buffalo randomly slaughtered for culture analysis and "all animals were culture negative for *B. abortus*."
- 1 female was euthanized due to "trauma at the handling facilities."
- 1 female died "unknown causes."
- + Unknown number of calves born in captivity 2010.
- + Unknown number of calves born in captivity 2011.

1 female from the 1st cohort has not calved, a requirement of the quarantine. "She may be released from quarantine after being spayed to ensure she does not get pregnant outside of the quarantine procedures."

Buffalo in quarantine at Slip N Slide: 68.

As you know, the Freedom of Information Act (FOIA) provides that if portions of a document are exempt from release, the remainder must be segregated and disclosed. We expect to receive all non-exempt portions of the documents that we have requested, and ask that you justify any deletions by reference to specific exemptions allowed under the FOI Act. The Buffalo Field Campaign reserves the right to appeal a decision to withhold any materials.

We hereby request a fee waiver for all search and duplication fees under the FOIA regulations [5 U.S.C. Sec. 552 (a) (4) (A) and 36 CFR 2.19(c) (1)]. The information requested will benefit the citizens of the United States and is for the purpose of public education and to encourage public debate on important policy issues. The requested information will be made available to the public through Buffalo Field Campaign's central office and/or our website.

Information available through the office and website is used in press conferences and releases, television and radio interviews, and regional and

national publications, and reaches a significant number of individuals nationwide, including through the following news sources: New York Times, Los Angeles Times, Washington Post, CNN, CBS, ABC, NBC, Headline News, London Times, UK Guardian, Japanese and German TV, National Geographic, PBS, Associated Press (nationally syndicated), Reuters (internationally syndicated), Planet Green Discovery Channel, Examiner, Indian Country Today, News from Indian Country, Bozeman Daily Chronicle, Helena Independent Record, Billings Gazette, Missoulian, Great Falls Tribune, West Yellowstone News, Livingston Enterprise, Montana Pioneer, Montana Standard, Flathead Beacon, Missoula Independent, Big Sky Weekly, Montana Public Radio, Pacifica Radio Stations, WBAI First Voices Indigenous Radio, KBZK-TV Bozeman, KXLF-TV Butte, ABC Montana, NBC Montana, CBS Montana, KGNU Colorado, Fox News Channel 8 Cleveland, Montana News Casper Star Tribune, Planet Jackson Hole, Jackson Hole News & Guide, Jackson Hole Weekly, Island Park News, Salt Lake Tribune, Powell Tribune, Ag Information Network, Idaho Statesman, Huffington Post, Word Press, New West, Yahoo! News, AlterNet, Mother Jones, Prairie Star, The Republic, Environmental News Service, Earth First! Journal, Mother Nature Network, CounterPunch, Animal People, Independent Media, multiple blogs and online news resources.

The language of the FOIA clearly indicates that Congress intended fees not to be a barrier to private individuals or public interest organizations seeking access to government records. In addition, the legislative history of the FOIA fee waiver language indicates that Congress intended a liberal interpretation of the phrase "primarily benefiting the public." This suggests that all fees are to be waived whenever the release of information contributes to public debate on important policy issues. This has been affirmed by the US Court of Appeals for the District of Columbia, in *Better Government Association v. Department of State*, 780 F. 2d 86 (D.C. Cir. 1986). In that case, the court found that under the FOIA, Congress had explicitly recognized the need for non-profit organizations to have free access to government documents and those government agencies cannot impair this free access by charging duplication or search for FOIA information requests. *Id.* at 89.

I appreciate your help and prompt response. Thank you for your time.

Sincerely,

/s/
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
406-646-0070
<http://www.buffalofieldcampaign.org>

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Rebecca K Frey](#); [Matt McCollum](#); [Pauline Nol](#)
Subject: Re: Fw: A quick idea to push "decreasing prevalence"
Date: Friday, February 12, 2010 5:25:00 PM
Attachments: [pic02021.gif](#)
[GnRH Bison Study Projected numbers.xlsx](#)

This sounds good! I made a spreadsheet to look at the numbers...taking into account repro failures, abortions, transmission,etc.....to get a feel for the numbers and to start thinking about which properties we would use.

(See attached file: GnRH Bison Study Projected numbers.xlsx)

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell
☐ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

02/10/2010 09:46 AM

ToPatrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA
cc

SubjectFw: A quick idea to push "decreasing
prevalence"


Here is a brief description of our proposed idea. What do you all think?

Jack

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 02/10/2010 09:44 AM -----

**Brian J
McCluskey/CO/APHIS/USDA**

02/05/2010 04:13 PM

ToJack C
Rhyan/CO/APHIS/USDA@USDA
ccMatt
McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA
SubjectRe: A quick idea to push "decreasing
prevalence" 

This is good, really good. I will visit with Dr. Clifford next week about refocusing our efforts on decreasing prevalence and about this project specifically.

Brian J. McCluskey, DVM, PhD, Dip. ACVPM
Director, Veterinary Services, Western Region
Fort Collins, CO
970.494.7385
☐ Jack C Rhyan/CO/APHIS/USDA

Jack C
Rhyon/CO/APHIS/USDA

02/05/2010 03:23 PM

To Brian J
McCluskey/CO/APHIS/USDA@USDA
cc Matt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA
Subject: A quick idea to push "decreasing prevalence"

Brian,

At our Starbucks brainstorm session on the way back to NWRC, we came up with this idea. Brogan's will be bison-free next week. In March Marty will start catching bison on the west side. We can collect 40 non pregnant heifers (seropositive and seronegative) and 4 bulls at the trap on the state ground and place them at Brogan's (or slip and slide) and begin a study to investigate what effect GnRH vaccine has on brucellosis transmission in YNP bison. In brief, after a period of several months' monitoring to find any seroconverting bison: Pasture A will contain 10 seropositive GnRH vaccinates, 10 seronegative nonvaccinates (sentinels) and 2 seronegative bulls. Pasture B will contain 10 seropositive non vaccinates, 10 seronegative nonvaccinates (sentinels) and 2 seronegative bulls. Over 3 years we will monitor calving and abortion results in all animals, and seroconversion to brucella seropositive in the sentinel groups. At the end of the study, we necropsy and culture the seropositive vaccinates and non vaccinates.

Hypothesis A: The use of GnRH vaccine reduces brucellosis transmission in bison.

Hypothesis B: Bison experiencing 3 years of anestrus have less brucella infection than normally cycling and calving bison (based on culture positive tissues and colony forming units per gram of tissue).

Hypothesis B is just something we have speculated about and this would be a perfect chance to test it. Also a perfect chance to test the Z nose in detecting brucellosis.

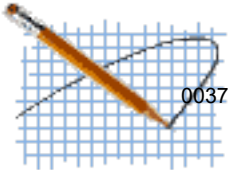
The best part of the study is the interpretive sign we put on the highway: **"Investigation of a contraceptive vaccine as a non lethal method of controlling populations and decreasing brucellosis prevalence in bison."** Also interviews we do with the news media, etc.

I ran it by Marty to see if he approved or not. He loves it. We could start it this spring. The NEPA issues for bison collection are already covered in the IBMP EIS. If we collect the bison on the west side we won't need YNP's blessing or research permit.

Down side: We have to keep the lease going a while longer. We will be dealing with hot brucella fetuses (We and Ryan and Becky are experienced with that). It'll set Suzanne's hair on fire.

What are your thoughts?

Jack



From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: Fw: A quick idea to push "decreasing prevalence"
Date: Tuesday, February 23, 2010 4:31:00 PM

heyI can't stop myself.....I'm on the A train to EXCEL hell.....

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ToPatrick R Clarke/MT/APHIS/USDA@USDA
ccMatt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA,


02/12/2010 04:09 PM

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SubjectRe: Fw: A quick idea to push "decreasing
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Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: Fw: A quick idea to push "decreasing prevalence"
Date: Sunday, February 14, 2010 12:28:00 PM
Attachments: [GnRH Bison Study Revised Projected numbers.xlsx](#)


Scary isn't it!!.....here is what is more disturbing.....Saturday afternoon that same normal vet is fixing a light fixture, his mind wanders, and he realizes the spreadsheet is all wrong.....he factored in that every calf was going to be a female. So on Sunday, it is bugging him so bad that he does the spreadsheet over.....pathetic really.

(See attached file: GnRH Bison Study Revised Projected numbers.xlsx)

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Date: Friday, February 12, 2010 4:08:00 PM

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Date: Tuesday, February 23, 2010 4:36:00 PM

I'll try to find you help. Now most guys would just write you off, but not me after all we've been thru, buddy, I'm there for you. (Besides, I'm worried about how you'll tarnish our good name.)


Thanks,

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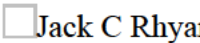
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Subject: A quick idea to push "decreasing prevalence"

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At our Starbucks brainstorm session on the way back to NWRC, we came up with this idea. Brogan's will be bison-free next week. In March Marty will start catching bison on the west side. We can collect 40 non pregnant heifers (seropositive and seronegative) and 4 bulls at the trap on the state ground and place them at Brogan's (or slip and slide) and begin a study to investigate what effect GnRH vaccine has on brucellosis transmission in YNP bison. In brief, after a period of several months' monitoring to find any seroconverting bison: Pasture A will contain 10 seropositive GnRH vaccinates, 10 seronegative nonvaccinates (sentinels) and 2 seronegative bulls. Pasture B will contain 10 seropositive non vaccinates, 10 seronegative nonvaccinates (sentinels) and 2 seronegative bulls. Over 3 years we will monitor calving and abortion results in all animals, and seroconversion to brucella seropositive in the sentinel groups. At the end of the study, we necropsy and culture the seropositive vaccinates and non vaccinates.

Hypothesis A: The use of GnRH vaccine reduces brucellosis transmission in bison.

Hypothesis B: Bison experiencing 3 years of anestrus have less brucella infection than normally cycling and calving bison (based on culture positive tissues and colony forming units per gram of tissue).

Hypothesis B is just something we have speculated about and this would be a perfect chance to test it. Also a perfect chance to test the Z nose in detecting brucellosis.

The best part of the study is the interpretive sign we put on the highway: **"Investigation of a contraceptive vaccine as a non lethal method of controlling populations and decreasing brucellosis prevalence in bison."** Also interviews we do with the news media, etc.

I ran it by Marty to see if he approved or not. He loves it. We could start it this spring. The NEPA issues for bison collection are already covered in the IBMP EIS. If we collect the bison on the west side we won't need YNP's blessing or research permit.

Down side: We have to keep the lease going a while longer. We will be dealing with hot brucella fetuses (We and Ryan and Becky are experienced with that). It'll set Suzanne's hair on fire.

What are your thoughts?

Jack

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Rebecca K Frey](#); [Pauline Nol](#); [Matt McCollum](#)
Subject: Re: FW: ACUC Proposal - signed
Date: Monday, May 16, 2011 4:47:00 PM

I've been having computer problems all day!!!.Sorry for the delay.
I like the document..... I will send it around to the ACUC, but will sign it now and FAX back the signature sheet to you. I especially like where the attending vet is "Patrick Rhyan Clarke" using the internationally accepted spelling of Rhyan.

Cheers,
Rhyan

P. Rhyan Clarke, D.V.M.
USDA/APHIS/VS
Regional Rhyanologist- GYA
Rhyanville, MT.
(406) 388-5162
(b) (6) -cell

 [Jack C Rhyan---05/16/2011 03:41:09 PM---From: Rhyan, Jack C \(APHIS\) Sent: Monday, May 16, 2011 12:08 PM](#)

From: Jack C Rhyan/CO/APHIS/USDA@MSOCOEX
To: Patrick R Clarke/MT/APHIS/USDA
Date: 05/16/2011 03:41 PM
Subject: FW: ACUC Proposal - signed

From: Rhyan, Jack C (APHIS)
Sent: Monday, May 16, 2011 12:08 PM
To: 'Clarke, Ryan P. (APHIS)'
Cc: Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS)
Subject: ACUC Proposal - signed

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If any of you want to change it, please let me know. I can make changes and resign and send it. Otherwise, Ryan, I think it is ready for the Committee's scrutiny.

Jack[attachment "ACUCGonaConBisonStudy.pdf" deleted by Patrick R Clarke/MT/APHIS/USDA]

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#)
Cc: [Rhyan, Jack C \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#)
Subject: Re: FW: ACUC Proposal - signed
Date: Tuesday, May 17, 2011 10:36:10 AM

Yhuck yhuck

From: Nol, Pauline (APHIS)
Sent: Tuesday, May 17, 2011 09:22 AM
To: Clarke, Patrick R. (APHIS)
Cc: Rhyan, Jack C (APHIS); Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS)
Subject: Re: FW: ACUC Proposal - signed

Rhyan,
I suspect you might be suffering from severe Rhyanitis and the current treatment for that is a Rhyanoplasty
Pauline

▼ Patrick R Clarke---05/16/2011 04:47:41 PM---I've been having computer problems all day!!!.Sorry for the delay.
I like the document.....

From: Patrick R Clarke/MT/APHIS/USDA
To: Jack C Rhyan/CO/APHIS/USDA@MSOCOEX
Cc: Rebecca K Frey/MT/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA, Matt
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Subject: RE: FW: ACUC Proposal - signed
Date: Tuesday, May 17, 2011 8:58:20 AM

Gimme back my h
Jack ryan

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To: Rhyan, Jack C (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
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To: [Jack C Rhyan](#)
Cc: [Brian J McCluskey](#); [Lowell A Miller/CO/APHIS/USDA](#); [Pauline Nol](#)
Subject: Re: Fw: brief protocol for bison immunocontraceptive project
Date: Friday, June 18, 2010 9:17:00 AM

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Jack C Rhyan/CO/APHIS/USDA 06/18/2010 09:05 AM	ToKathleen A Fagerstone/CO/APHIS/USDA@USDA ccLowell A Miller/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA, Brian J McCluskey/CO/APHIS/USDA@USDA SubjectFw: brief protocol for bison immunocontraceptive project
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Thanks,

Jack

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 06/18/2010 09:01 AM -----

Jack C Rhyan/CO/APHIS/USDA 06/18/2010 08:53 AM	ToKathleen A Fagerstone/CO/APHIS/USDA ccLowell A Miller/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA, Brian J McCluskey/CO/APHIS/USDA@USDA SubjectRe: Fw: brief protocol for bison immunocontraceptive project 
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--	---

06/18/2010 08:14 AM

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SubjectRe: Fw: brief protocol for bison
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What do you think?

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**Lowell A
Miller/CO/APHIS/USDA**

ToKathleen A
Fagerstone/CO/APHIS/USDA@USDA

cc

06/17/2010 04:55 PM

SubjectFw: brief protocol for bison
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FYI

Lowell A. Miller Ph.D,
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone (970)266-6163
Fax (970)266-6157
e-mail-Lowell.A.Miller@aphis.usda.gov

----- Forwarded by Lowell A Miller/CO/APHIS/USDA on 06/17/2010 04:25 PM -----

**Jack C
Rhyan/CO/APHIS/USDA**

06/16/2010 12:36 PM

ToBrian J
McCluskey/CO/APHIS/USDA@USDA,
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Thanks much.

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[attachment "ImmunocontBisonProject.doc" deleted by Kathleen A
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Subject: Re: Fw: brief protocol for bison immunocontraceptive project
Date: Friday, June 18, 2010 8:53:00 AM
Attachments: [ImmunocontBisonProject.doc](#)

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Jack

(See attached file: *ImmunocontBisonProject.doc*)

Proposed Project:

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites

2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

This general research plan will be followed; details will be worked out in further consultation with collaborators and a more extensive protocol developed. A total of approximately 46 yearling bison (approximately half seropositive and half seronegative females and 6 seronegative males) captured in winter/spring as part of the ongoing Interagency Bison Management Plan will be transported to the bison quarantine feasibility study facilities in Corwin Springs, Montana. Seronegative animals will be separated from seropositives and monitored monthly by serology until August. Bulls will be maintained separately and monitored by serology. In August, animals will be relocated into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive GonaCon™ vaccine and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female sentinel bison will be fitted with proximity collars programmed to record proximity to one another and to transmitters on vaginal implants. Over the 3 year period, calving, abortion results, and serology in the groups will be monitored. In February each year animals will be pregnancy tested and pregnant animals fitted with vaginal transmitters. Transmitters will alert investigators to abortion or calving events and record exposure of sentinel animals. Animals will be tested by serology in February and in summer following calving. At the end of the study, necropsy and culture of all adult animals will occur. Offspring from the study will be monitored by serology and culture twice a year throughout the study. Offspring that remain or become positive for *B. abortus* by serology or culture after weaning will be euthanized and necropsied. Offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be translocated to tribal and/or public lands.

Expected outcomes:

1. Determine the effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds.
2. Determine the effect prolonged anestrus has on the transmission of *B. abortus* in bison herds.
3. Determine the risk and extent of exposure of bison herd members to *B. abortus* at parturition sites.
4. Determine nature of infection in calves due to suckling of seropositive cows.
5. Determine risk of venereal transmission of *B. abortus* to seronegative bull bison.

From: [Jack C Rhyan](#)
To: [Kathleen A Fagerstone](#)
Cc: [Brian J McCluskey](#); [Lowell A Miller/CO/APHIS/USDA](#); [Pauline Nol](#)
Subject: Re: Fw: brief protocol for bison immunocontraceptive project
Date: Friday, June 18, 2010 9:23:00 AM

I used to do that in the Yellowstone area too, in fact Jack used to write the CatEx for me. But now when I ask Jack whether or not we can do it, he says, "Hmmm, I think we'd better do an EA." Crazy. We'll talk.

Jack

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Fort Collins, CO 80521

Phone (970)266-6163

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e-mail-Lowell.A.Miller@aphis.usda.gov

----- Forwarded by Lowell A Miller/CO/APHIS/USDA on 06/17/2010 04:25 PM -----

**Jack C
Rhyan/CO/APHIS/USDA**

06/16/2010 12:36 PM

To Brian J

McCluskey/CO/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA,
Lowell A Miller/CO/APHIS/USDA@USDA,
(b) (6) @gmail.com>

cc Pauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA

Subject: brief protocol for bison immunocontraceptive
project

All,

Attached is a brief protocol I'd like to send to Jack Edmundson for them to start work on. It will undoubtedly require an EA with public meetings. This will be enough for them to start with. Please review and correct, expand, etc.

Thanks much.

Jack

[attachment "ImmunocontBisonProject.doc" deleted by Kathleen A
Fagerstone/CO/APHIS/USDA]

From: [Brian J. McCluskey](#)
To: [Rebecca K. Frey](#)
Cc: [Pauline Nol](#); [Jack C. Rhyan](#)
Subject: Re: Fw: GonaCon comments
Date: Monday, March 14, 2011 3:08:00 PM
Attachments: [ATTMDM15.doc](#)

This is great that the Park Service is engaging to this degree. Please let's find every opportunity to work with them to design a scientifically sound, statistically valid project. At this point I would rather not let funding drive any of the decisions. Please move ahead and design the study that is going to be defensible, figure out what that will cost and we will go from there. If I need to touch base with YNP to initiate more dialogue or for anything else please let me know.

Brian

Brian J. McCluskey, DVM, MS, PhD, Dip. ACVPM
Director, Veterinary Services, Western Region
Fort Collins, CO
970.494.7385

☐ Rebecca K Frey/MT/APHIS/USDA

**Rebecca K
Frey/MT/APHIS/USDA**

To: Brian J McCluskey/CO/APHIS/USDA
cc

Subject: Fw: GonaCon comments

03/14/2011 12:36 PM

Hi,

We got back some comments from NPS on our GonaCon study. I have attached it here for you to review. The most important comments are in regards to sample size. The main question we need to answer right away is, how many should we begin with to have an adequate sample size? This will affect how many I continue to sort into the study group, as well as what we need to get from the positive pen later. We can look at it 2 ways, 1) get a larger group to begin with and have a good plan for pulling off calves that can go immediately into the quarantine procedure, or 2) keep calves from a smaller initial sample size and vaccinate them as they become eligible to increase our sample size. I have asked Kammy to look into our sample size as well, to see how many we really need for power. This then all comes down to budget to feed and house bison in order to complete this study. We can certainly accommodate some larger numbers, but will need to seriously consider how long to keep these animals and what to do with calves born to the study.....

Rick offered to actually do our permit application, since he knows the system and could do it quickly.

Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist

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Bozeman, Montana
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----- Forwarded by Rebecca K Frey/MT/APHIS/USDA on 03/14/2011 12:31 PM -----

Pauline
Nol/CO/APHIS/USDA

ToRebecca K Frey/MT/APHIS/USDA@USDA
cc

Subjectplot gets thicker.....

03/09/2011 02:47 PM

Hey Becky,

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They were very concerned about the numbers being used, and I have to agree, since the magic number 10 isn't all that magical and we need to have more substance behind our choice of sample size. I am working on that but probably need help on that. The one main thing is that I don't know what our space and financial limits are. Would you be able to give a fixed limit on numbers of animals we can have in the pastures and what we can afford to feed? I don't even know how we are paying for this study.

There are many comments on wanting to carry the animals out further than what the protocol now suggests, which would be great to evaluate the vaccine, but don't know how financially or logistically feasible this would be. Maybe NPS could chip in if they really want to get those data points???

Lastly, I left a message with Rick Wallen to call me. Jenny was frankly surprised to hear that animals were being moved around at this point and so I was wanting to find out Rick's take on how urgent this protocol is to push through in relation to being able to keep these animals. You may have already told me this but, as I said, give me 15 minutes, and it's amazing what I forget.

This has the potential of being a very good, but much more complicated study than it started out as. I feel that if we are going to put the effort into it, we should learn as much as we can, and answer NPS's questions, since they are the people we are trying to convince to use the vaccine, if it looks feasible. But, this can become a huge monster too.

I have to go pick up kiddies now but I will work some more on this tomorrow.
Pauline

(See attached file: Rhyan Immuncontraception Study Plan_rlw review brmd.doc)

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing ~~potential for transmission~~ shedding of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS: Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria occurrence of following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing transmission the shedding of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission shedding in a bison herd

Commented [MAW1]: Transmission is not addressed here. Change to "potential for transmission" or "shedding"

Commented [MAW2]: Generally, the concept is unique and interesting. Proposal requires some more critical review and statistical input prior to implementation.

Commented [MAW3]: Then is the sample size proposed sufficient to address this question?

Commented [MAW4]: See comment on title

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Commented [MAW5]: How will you determine colonization? Is this once 3 yr post-vaccination? How much natural variation would you expect to see?

Commented [MAW6]: Excellent point. Study should continue after contraceptive effects have been lost.

3. Determine the effect of immune system stimulation via vaccination with GonaCon adjuvant on brucella titers and shedding.

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Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites.
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison, looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Commented [MAW7]: Granted the sample size is too small to do this, but it is a very important point. What is the potential for confounding effects of local inflammatory response from the adjuvant. Without adjuvant vaccinated controls, this can't be determined.

Commented [MAW8]: This portion of the study is not fully developed enough to know whether this objective could be met

Commented [MAW9]: Power?

Commented [MAW10]: Power?

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive – 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated.

Commented [MAW11]: How was this n selected? Will it allow adequate power based on expected outcomes?

Commented [MAW12]: What if the adjuvant has an effect? Lack of a group of adjuvant treated will result in confounding of results. Are results of "vaccination" from lack of reproduction or could they be confounded with immune stimulation? This design issue needs to be addressed before implementing this project.

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be ~~used~~ made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Commented [r13]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r14]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r15]: Why not get the culture done as soon as possible?

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined, preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector
↳ Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting? I'm not sure if this is answering a behavioral question (do bison investigate aborted fetuses) or a disease question (how often do aborted fetuses initiate seroconversion).
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy whether they would have an infectious live birth or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

Commented [jgp16]: Would be helpful to have a table of response variables, estimated variation expected between treatment groups, and what question they are meant to answer.

Commented [jgp17]: Is this measured through differences in proportion of seroconversion between the pastures, proportion culture positive, proportion with culture positive abortions, or ?? If using seroconversion as the marker what proportion of seroconversion would you expect on a yearly basis with exposure to seropositive females?

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Commented [jgp18]: Does this mean that we expect all animals to be seronegative at the end of the study or that no new ones will have seroconverted. This seems like it is directly related to the first outcome.

Commented [MAW19]: Are they infected or seropositive?

Commented [MAW20]: How will you do this and is power sufficient?

Commented [jgp21]: How do you rule out in-utero transmission?

Commented [MAW22]: This, like many of the other outcomes, seem to be overstated a bit. Some insights will no doubt be gained, but would like to see more evidence that data will actually allow "determination" of these factors.

Commented [MAW23]: Power? Pretty small sample of bulls, particularly if not all are actively breeding.


From: [Jack C Rhyan](#)
To: [Brian J McCluskey](#)
Cc: [Pauline Nol](#)
Subject: Re: Fw: GonaCon comments
Date: Friday, March 25, 2011 10:29:00 AM

Brian,

Here is what we are thinking. We enlarge the study to accomodate the Park's ideas and see if they can pick up the Brogan lease (they have already expressed interest in that) and become a partner in the work. We can then put the Brogan lease money into Rigler's and Slip and Slide and we'll have enough space to do it right. Then after working together on this project, if the contraceptive idea works, it becomes the Park Services' idea. Perfect!

What do you think?

Jack

 [Brian J McCluskey---03/14/2011 03:13:48 PM---This is great that the Park Service is engaging to this degree. Please let's find every opportunity to work with them to desig](#)

**Brian J
McCluskey/CO/APHIS/USDA**

03/14/2011 03:08 PM

ToRebecca K
Frey/MT/APHIS/USDA@USDA
ccPauline Nol/CO/APHIS/USDA@USDA,
Jack C
Rhyan/CO/APHIS/USDA@USDA
SubjectRe: Fw: GonaCon comments



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Brian

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Director, Veterinary Services, Western Region
Fort Collins, CO
970.494.7385

 [Rebecca K Frey/MT/APHIS/USDA](#)

**Rebecca K
Frey/MT/APHIS/USDA**

03/14/2011 12:36 PM

ToBrian J McCluskey/CO/APHIS/USDA
cc
SubjectFw: GonaCon comments

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need to answer right away is, how many should we begin with to have an adequate sample size? This will affect how many I continue to sort into the study group, as well as what we need to get from the positive pen later. We can look at it 2 ways, 1) get a larger group to begin with and have a good plan for pulling off calves that can go immediately into the quarantine procedure, or 2) keep calves from a smaller initial sample size and vaccinate them as they become eligible to increase our sample size. I have asked Kammy to look into our sample size as well, to see how many we really need for power. This then all comes down to budget to feed and house bison in order to complete this study. We can certainly accommodate some larger numbers, but will need to seriously consider how long to keep these animals and what to do with calves born to the study.....

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Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell

----- Forwarded by Rebecca K Frey/MT/APHIS/USDA on 03/14/2011 12:31 PM -----

Pauline
Nol/CO/APHIS/USDA

ToRebecca K Frey/MT/APHIS/USDA@USDA
cc

Subjectplot gets thicker.....

03/09/2011 02:47 PM

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I have to go pick up kiddies now but I will work some more on this tomorrow.
Pauline

From: Patrick R Clarke <patrick.r.clarke@aphis.usda.gov>
 Sent: Friday, February 12, 2010 3:25 PM
 To: Jack C Rhyan
 Cc: Rebecca K Frey; Matt McCollum; Pauline Nol
 Subject: Re: Fw: A quick idea to push "decreasing prevalence"
 Attachments: pic02021.gif; GnRH Bison Study Projected numbers.xlsx

This sounds good! I made a spreadsheet to look at the numbers...taking into account repro failures, abortions, transmission, etc.....to get a feel for the numbers and to start thinking about which properties we would use.

(See attached file: GnRH Bison Study Projected numbers.xlsx)

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 USDA/APHIS/VS
 Regional Epidemiologist- GYA
 Belgrade, MT.
 (406) 388-5162
 (b) (6) -cell
 Jack C Rhyan/CO/APHIS/USDA

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Patrick R Clarke/MT/APHIS/USDA@USDA, Rebecca K
 Frey/MT/APHIS/USDA@USDA

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Fw: A quick idea to push "decreasing prevalence"

Here is a brief description of our proposed idea. What do you all think?
 Jack

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 02/10/2010 09:44 AM -----

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Matt McCollum/CO/APHIS/USDA@USDA, Pauline
Nol/CO/APHIS/USDA@USDA

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Re: A quick idea to push "decreasing prevalence"

This is good, really good. I will visit with Dr. Clifford next week about refocusing our efforts on decreasing prevalence and about this project specifically.

Brian J. McCluskey, DVM, PhD, Dip. ACVPM
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970.494.7385
Jack C Rhyan/CO/APHIS/USDA

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A quick idea to push "decreasing prevalence"

Brian,
At our Starbucks brainstorm session on the way back to NWRC, we came up with this idea. Brogan's will be bison-free next week. In March Marty will start catching bison on the west side. We can collect 40 non pregnant heifers (seropositive and seronegative) and 4 bulls at the trap on the state ground and place them at Brogans (or slip and slide) and begin a study to investigate what effect GnRH vaccine has on brucellosis transmission in YNP bison. In brief, after a period of several months' monitoring to find any seroconverting bison: Pasture A will contain 10 seropositive GnRH vaccinates, 10 seronegative nonvaccinates (sentinels) and 2 seronegative bulls. Pasture B will contain 10 seropositive non vaccinates, 10 seronegative nonvaccinates (sentinels)

and 2 seronegative bulls.

Over 3 years we will monitor calving and abortion results in all animals, and seroconversion to brucella seropositive in the sentinel groups

At the end of the study, we necropsy and culture the seropositive vaccinates and non vaccinates

Hypothesis A: The use of GnRH vaccine reduces brucellosis transmission in bison.

Hypothesis B: Bison experiencing 3 years of anestrus have less brucella infection than normally cycling and calving bison (based on culture positive tissues and colony forming units per gram of tissue).

Hypothesis B is just something we have speculated about and this would be a perfect chance to test it. Also a perfect chance to test the Z nose in detecting brucellosis.

The best part of the study is the interpretive sign we put on the highway: "Investigation of a contraceptive vaccine as a non lethal method of controlling populations and decreasing brucellosis prevalence in bison." Also interviews we do with the news media, etc.

I ran it by Marty to see if he approved or not. He loves it. We could start it this spring. The NEPA issues for bison collection are already covered in the IBMP EIS. If we collect the bison on the west side we won't need YNP's blessing or research permit.

Down side: We have to keep the lease going a while longer. We will be dealing with hot brucella fetuses (We and Ryan and Becky are experienced with that). It'll set Suzanne's hair on fire.

What are your thoughts?

Jack

From: [Nol, Pauline - APHIS](#)
To: [Eickholt, Donita S - APHIS](#); [Bruce, Samantha - APHIS](#)
Subject: RE: FYI: Trip reports
Date: Monday, June 22, 2015 9:15:00 AM
Attachments: [Trip Report - Nol Gardiner MT June 2015.doc](#)

Hi Donita,
Here is my trip report.
Thanks!
Pauline

From: Eickholt, Donita S - APHIS
Sent: Monday, June 22, 2015 7:26 AM
To: Nol, Pauline - APHIS; Bruce, Samantha - APHIS
Subject: FYI: Trip reports

Please fill out trip report and submit to me electronically.

I am finally able to process your Vouchers. (this new travel system has been back logged).

If this can get done today, I can push this through.

Thanks!

Donita S. Eickholt
Program Assistant
NVSL/DBL/MB
1920 Dayton Avenue
Ames, IA 50010
Phone: 515 337 7388

National Veterinary Services Laboratories	
Document Title: Summary Report Form for Trips, Meetings, Committees, and Working Groups	
Author/Position: Nancy Clough, Chief of Staff	Document Number: FMA-NVSL-0001.03
Page 1 of 2	Supersedes: NVSLFMAMR01.02

Summary Report Form for Trips, Meetings, Committees, and Working Groups

Pauline Nol (Wildlife Epidemiologist) and Samantha Bruce (Saul Wilson Scholar) travelled to Gardiner, MT on June 7, 2015. On June 8, 9, and 10 Dr. Nol and Ms. Bruce assisted with bison handling and sample collection from bison being held at the Bison Quarantine Facility in Corwin Springs, MT. This work is a required component of the protocol associated with the study entitled “Evaluation of GonaConTM, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison”. . Dr. Nol and Ms. Bruce travelled back to Fort Collins, CO on June 10, 2015. This study is part of WiLDIT’s research on management of diseases at the wildlife-livestock interface.

Approved: /s/ Paul Hauer

Summary Report Form for Trips, Meetings, Committees, and Working Groups

Name: Pauline Nol

Purpose/Function: Travel to Gardiner, MT to assist with bison handling and sample collection related to the following study: "Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison". Bison are being held at the Bison Quarantine Facility in Corwin Springs, MT

Date(s): June 7-10, 2015

Location: Gardiner, MT

Meeting Title: NA

Participant(s)/Attendees: NA

Bullet point summary of meeting topics of possible interest/impact to NVSL: NA

Feedback requested from Director's Office: NA

Meeting proceedings/notes attached: Yes ___ No X

Summary report* submitted to travel clerk with voucher information: Yes X No ___

*Required to be submitted with travel voucher within 5 business days of return. If no voucher is involved, submit directly to supervisor and Lead Secretary within 3 business days of meeting.

From: [Frey, Rebecca K \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#)
Cc: [Rhyan, Jack C \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: Re: GnRH Conference call
Date: Tuesday, May 24, 2011 10:30:47 AM

My 2 cents are yes, we have made some significant changes, and should probably talk about future work needs. We can have a quickie with the park if you like and then stay on to discuss operations.
B

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

▼ Matt McCollum/CO/APHIS/USDA@MSOCOEX

**Matt
McCollum/CO/APHIS/USDA@MSOCOEX**

05/24/2011 09:59 AM

ToJack C
Rhyan/CO/APHIS/USDA,
Pauline
Nol/CO/APHIS/USDA,
Rebecca K
Frey/MT/APHIS/USDA,
Patrick R
Clarke/MT/APHIS/USDA
cc
SubjectGnRH Conference call

Do we need to set another one up?

From: [Laura B Greiner](#)
To: [Pauline Nol](#)
Cc: [Catherine M Bens](#)
Subject: Re: GnRH protocols
Date: Wednesday, March 02, 2011 8:47:00 AM
Attachments: [test system = bison.rtf](#)
[test substance = GnRH.rtf](#)
[Search = GnRH.rtf](#)

Pauline,

I've attached three searches relating to GnRH and bison. Send me an email when you are ready to be assigned a QA number.

Laura

(See attached file: test system = bison.rtf)(See attached file: Search = GnRH.rtf)(See attached file: test substance = GnRH.rtf)

☐ Pauline Nol---03/02/2011 08:40:42 AM---Hi there, I'm putting together a protocol for a GnRH study in bison and I don't have access to related protocols (Section 6) s

**Pauline
Nol/CO/APHIS/USDA**

ToCatherine M Bens/CO/APHIS/USDA@USDA,
Laura B Greiner/CO/APHIS/USDA@USDA

cc

03/02/2011 08:40 AM

SubjectGnRH protocols

Hi there,

I'm putting together a protocol for a GnRH study in bison and I don't have access to related protocols (Section 6) since I assume they are on the intranet?? Would you be able to give me a list of previous NWRC GnRH protocols related to bison or other ungulates? Or let me know how to access that list?

Thanks!

Pauline

NWRC STUDY RECORDS
(Test System = 'Bison')
(March 2, 2011)

<i>Study Director</i>	<i>QA Number</i>	<i>Title</i>
Phillips	1371	Automated species recognition system for controlling animal access to resources: calibration and evaluation for North American species

NWRC STUDY RECORDS
(Test Substance = 'GnRH')
(March 2, 2011)

<i>Study Director</i>	<i>QA Number</i>	<i>Title</i>
Felix	533	Use of attenuated Salmonella typhimurium as a live vector for the oral delivery of immunocontraceptive vaccines
Mathies	939	Potential of gonadotropin-releasing hormone antigen for ophidian immunocontraception
	1075	Evaluation of five agents for inhibiting reproduction in the Brown Tree Snake
	1233	Effect of a GnRH vaccine on male brown tree snake reproduction
Miller	451	Development of immunocontraception technology to control reproduction in the coyote (Canis latrans)
	579	Oral contraceptives for the Norway rat
	1396	NWRC adjuvant efficacy testing
Nash	871	Efficacy tests in rabbits for adjuvant and antigen formulations in infertility immunization
	871	Efficacy tests in rabbits for adjuvant and antigen formulations in infertility immunization
	911	Test of GonaCon (GnRH vaccination) treatment in an urban Berkeley, California population of California ground squirrels as a population management tool
Perry	1216	Chemical sterilization of black-tailed deer
Powers	933	Evaluation of Adjuvac emulsion as an alternative to Freund's complete and incomplete adjuvant as a carrier for GNRH-KLH vaccine
Yoder	508	Development and comparison of immunocontraceptive techniques and a competitive cholesterol inhibition technique for control of avian reproduction
	1062	Development of a vaccination protocol for immunization of Japanese Quail

NWRC STUDY RECORDS

(Title contains 'GnRH')

(March 2, 2011)

<i>Study Director</i>	<i>QA Number</i>	<i>Title</i>
Campbell	1549	Chemical sterilization of captive male shoats with a GnRH vaccine
	1783	Oral vaccination of feral swine with a GnRH vaccine
Kemp	1601	Efficacy testing of new GnRH peptide lots, adjuvant formulation changes for GonaCon production, and a novel French Immunocontraceptive protein
	1696	Bioassay to evaluate the efficacy of a recombinant anti-GnRH protein
	1786	Oral delivery of Salmonella choleraesuis vaccine vector expressing a recombinant multimeric GnRH protein
Mathies	1233	Effect of a GnRH vaccine on male brown tree snake reproduction
Mauldin	1769	Porcine Tonsillar and Ileal uptake and transport of oral vaccines: GnRH, Mycobacterium avium, BCG, and RB-51
Miller	1287	Efficacy and comparison of vitrified injectable and vitrified oral GnRH immunocontraception of rabbits
Nash	911	Test of GonaCon (GnRH vaccination) treatment in an urban Berkeley, California population of California ground squirrels as a population management tool
Powers	933	Evaluation of Adjuvac emulsion as an alternative to Freund's complete and incomplete adjuvant as a carrier for GNRH-KLH vaccine
Yoder	1382	Effect of GnRH vaccine on black-tailed prairie dogs
	1563	Transdermal application of a recombinant GnRH vaccine

From: [Laura B Greiner](#)
To: [Pauline Nol](#)
Subject: Re: GnRH protocols
Date: Wednesday, March 02, 2011 9:09:00 AM
Attachments: [AD003-04 TEMPLATE complete protocol and all appendices Rev 2-18-11.docx](#)
[AD003-04 Study Protocols.pdf](#)

Pauline,

QA-1858. Attached is the updated protocol template and the SOP that describes it. Please contact me or Cathy with any questions.

Laura

(See attached file: AD003-04 Study Protocols.pdf)(See attached file: AD003-04 TEMPLATE complete protocol and all appendices Rev 2-18-11.docx)

☐ Pauline Nol---03/02/2011 08:54:58 AM---Thank you Laura! I might as well get a QA number now. The title is: Evaluation of GonaCon™, an immunocontraceptive vaccine,

**Pauline
Nol/CO/APHIS/USDA**

03/02/2011 08:54 AM

ToLaura B Greiner/CO/APHIS/USDA@USDA
ccCatherine M Bens/CO/APHIS/USDA@USDA

SubjectRe: GnRH protocols



Thank you Laura! I might as well get a QA number now.

The title is: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of Brucella abortus in bison

And the study director is Jack Rhyan

I believe this is going to be GLP and treated in a similar manner to the elk study up in RMNP

United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center
PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Writing or collaborating on review papers and synthesis reports Student committee participation Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Training programs requiring the use of animals Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Collaborating on study design, data analysis, or economic analysis. Minor participation on a regulated study at the collaborating host institution A study that does not include animal use, etc.
4 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> A typical NWRC led study Major NWRC staff participation in regulated activity Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:

_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☐ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact: _____

☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART THREE: DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☐ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☐ *Data analysis, interpretation and reporting*
 - ☐ *Other: _____*

Collaboration:	Name	Address or Organization	Role in Project

Start Date:

End Date:

Archive Date:

- Anticipated Project Outcome:
- ☐ Manuscript
 - ☐ Report
 - ☐ Other: _____

Materials to be archived to close this activity:

Description of Project and NWRC Activities and Participation:

Comments:

Attachments:
(e.g. Material Transfer Form, IACUC approval, etc.)

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Other Investigators, Collaborators, Cooperators, and Consultants		

2. Testing Facilities

Name	Address	Role in Study

3. Sponsor

Name	Address	Contract No.

4. Schedule

Proposed Experimental Start Date:
Proposed Experimental Termination Date:
Proposed Study Completion/Archive Date:

5. Background and Justification

Give the rationale for the study with an analysis of the problem situation and a clear statement of need and justification. Include a summary of the literature reviewed.

6. Related Protocols

List by Protocol Number

7. Assurance of Non-Duplication of Studies

Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.

8. Objective/Hypotheses

Give concise statements as to the objective of the study and the hypotheses to be tested.

9. Methods/Procedures

Give a logical sequence of events leading toward attainment of the objectives including the type and frequency of tests, measurements, and analyses to be made. The level of detail should be at a level which would allow an independent third party or educated lay person to read and conceptually understand it and a scientific researcher to conduct or repeat the study based solely on the protocol. For field studies include a description of the field sites where the study will be conducted. Refer to details in the attached appendices as appropriate. Analytical chemistry procedures may be indicated in the attached appendices, but all other methods and procedures must be provided directly or by reference to the appropriate SOP(s). Information frequently forgotten includes randomization schemes and procedures, bioanalytical assays, and a comprehensive description of all procedures and methods (field and lab), etc.

10. Experimental Design and Statistical Analyses

Describe the experimental design including methods for control of bias. Include sample sizes, sketches, and narrative as needed to make the design clear. Give a statement of the proposed statistical method or methods to be used. If a statistician was consulted for assistance in study design, give the date of the consultation and the name and affiliation of the person consulted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a.
 - b.
 - c.
 - d.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx		
A. Salary and Benefits					
B. Facilities (in addition to existing facility or space costs)					
C. Equipment					
D. Supplies					
E. Animal Care Costs					
F. Operating Costs (travel, misc. services, etc)					
TOTAL	\$0	\$0	\$0		

14. Human Health and Safety

Cite the appropriate SOP(s) or explain briefly the safety precautions, equipment, and procedures to be used for potentially hazardous conditions. State whether or not the proposed research has any potential for risk to the health or safety to members of the public, and, if so, explain how such risk(s) will be minimized or avoided.

15. Staff Qualifications

[Standard text revise as needed] All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study.

16. Archiving

[Standard text revise as needed] All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

[Standard text revise as needed] Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

List in alphabetical order by author.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☐ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☐ NEPA and ESA Appendix
 - ☐ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: (include appropriate title) _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An “Animal” is defined as any vertebrate. “Use” includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal Description

1) Animals:

Species, subspecies (if applicable):

Breed, strain and substrain (if applicable):

Total Number and Sex:

Body weight range:

Age:

B. Rationale for involving animals, for appropriateness of species, and for numbers Provide justification why this study requires the use of animals, and for the numbers to be used.

1) Rationale for involving animals:

2) Rationale for appropriateness of the species to be used:

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):

C. Source

Describe where the animals will be trapped or obtained, or identify the vendor by name and address.

D. Method of identification of animals

Cite the appropriate SOP(s) or explain briefly how animals will be marked or identified to prevent misidentification.

E. Trapping/Collecting

Cite the appropriate SOP(s) or explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.)

F. Transport

Cite the appropriate SOP or explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, etc.

G. Handling/restraint

Cite the appropriate SOP(s) or explain briefly how the animals will be held or restrained (manual vs. chemical) throughout study.

H. Quarantine

Cite the appropriate SOP, or describe the procedure for the quarantine of animals.

I. Housing/maintenance

Cite the appropriate SOP(s) or explain briefly how housing/maintenance will be done (including information on feeder animals if used).

J. Dietary contaminant exposure

Are there any contaminants or diet supplements that are reasonably expected to be present in the dietary materials, drinking water, or bedding material and are known to be capable of interfering with the purpose or conduct of the study? If so, please describe control/testing mechanism.

K. Disposition of animals

Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study, or cite the appropriate SOP(s).

L. Animal pain or distress**1) Consultation with Attending Veterinarian:**

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: _____

Date of Consultation: _____

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☐ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

M. Euthanasia

Describe the appropriate method of euthanasia to be used (cite the appropriate SOP or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)

N. IACUC Approval

Date of IACUC Approval Letter: _____

O. Staff Qualifications

List the study participants that will be working independently with animals and provide their qualifications/certifications (i.e. name, title, and a brief description of training/experience).

Analytical Chemistry Appendix

If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

- A. Number of samples to be analyzed (by type):**
- B. Storage conditions (temperature, container type, light/dark, duration):**
- C. Method title and number:**
- D. ACP Leader approval: _____ Date: _____**
(attach email or letter of concurrence from Analytical Services Project Team Leader)

If chemical analysis will be made by a laboratory outside of NWRC, include A-C above and attach the method to be used.

Column E Explanation

1. Registration Number: 84-F-0001
2. Number of animals used in this study during this reporting period:
3. Species (common name) of animals used in study during this reporting period:
4. Explain procedure producing pain and/or distress:
5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):
6. What, if any, federal regulations require this procedure?

Agency: CFR:

Material Transfer Agreement

STANDARD AGREEMENT
U. S. Department of Agriculture
Animal and Plant Health Inspection Service
National Wildlife Research Center

PARTIES:

APHIS: USDA, APHIS
 National Wildlife Research Center
 Scientist Address
 City, State Zip
 Tel: Telephone # of Scientist
 FAX: FAX # of Scientist
 E-Mail: E-mail address of Scientist

Recipient: Company Name
 Company Address
 City, State Zip of Company
 Tel: Telephone # of Recipient
 FAX: FAX # of Recipient
 E-mail: E-mail address of Recipient

PURPOSE:

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

[Table may be adjusted as needed]

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material, shall provide APHIS with any manuscript that describes the work with the Material and shall acknowledge APHIS' contribution to the work reported when appropriate.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's

use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.

7. Upon completion of the activities performed using the Material, the Material shall be [redacted] *[for example, returned to ..., destroyed by, disposed of as instructed by APHIS].*

8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated].* THIS MATERIAL MAY BE INFECTED WITH PATHOGENS INCLUDING, BUT NOT LIMITED TO, *[NAME OF PATHOGEN]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.

9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed of.

QA#:	Permit Information (Type and Number):
------	--

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:

_____ Typed name/Title	_____ Signature (NWRC Scientist)	_____ Date
_____ Typed Name/Title	_____ Signature (NWRC Project Leader)	_____ Date

APHIS APPROVING OFFICIAL:

_____ Typed Name/Title	_____ Signature (NWRC Assistant Director)	_____ Date
---------------------------	--	---------------

ACCEPTED FOR RECIPIENT:

_____ Typed Name/Title	_____ Signature	_____ Date
---------------------------	--------------------	---------------

Original: Quality Assurance Unit

Material Transfer Agreement

ANIMAL / ANIMAL TISSUE TRANSFER AGREEMENT

U. S. Department of Agriculture
Animal and Plant Health Inspection Service
National Wildlife Research Center

PARTIES:

APHIS: USDA, APHIS
National Wildlife Research Center
Scientist Address
City, State Zip
Tel: Telephone # of Scientist
FAX: FAX # of Scientist
E-Mail: E-mail address of Scientist

Recipient: Company Name
Company Address
City, State Zip of Company
Tel: Telephone # of Recipient
FAX: FAX # of Recipient
E-mail: E-mail address of Recipient

PURPOSE:

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

[Table may be adjusted as needed]

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material, shall provide APHIS with any manuscript that describes the work with the Material and shall acknowledge APHIS' contribution to the work reported when appropriate.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.

6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.

7. Upon completion of the activities performed using the Material, the Material shall be [redacted] *[for example, returned to ..., destroyed by, disposed of as instructed by APHIS].*

8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated].* THIS MATERIAL MAY BE INFECTED WITH PATHOGENS INCLUDING, BUT NOT LIMITED TO, *[NAME OF PATHOGEN]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.

9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed of.

QA#:	Permit Information (Type and Number):
------	--

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:

_____ Typed name/Title	_____ Signature (NWRC Scientist)	_____ Date
_____ Typed Name/Title	_____ Signature (NWRC Project Leader)	_____ Date

APHIS APPROVING OFFICIAL:

_____ Typed Name/Title	_____ Signature (NWRC Assistant Director)	_____ Date
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ACCEPTED FOR RECIPIENT:

_____ Typed Name/Title	_____ Signature	_____ Date
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Original: Quality Assurance Unit

Microbiological/Biohazardous Materials Use Appendix

NWRC proposed research or testing activities which involve the use of microbiological organisms or biohazardous agents at or above a Biosafety Level 2 or Risk Level 2, or use recombinant DNA *in vivo*, require this appendix to be completed and submitted to the NWRC IBC for review and approval.

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at www.cdc.gov/od/ohs/biosfty/biosfty.htm for the definitions and lists of BioSafety Level 2 organisms and above.

Reference the American Biological Safety Association's (ABSA) "Risk Group Classification for Infectious Agents" at <http://www.absa.org/resriskgroup.html> for the definitions and lists of Risk Level 2 agents and above.

Reference the National Institute of Health's (NIH) Guidelines for Recombinant DNA and Gene Transfer at www4.od.nih.gov/oba/rac/documents1.htm for specific practices for constructing and handling recombinant DNA and organisms/viruses containing recombinant DNA molecules. Definition of recombinant DNA; 1) Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or 2) Molecules that result from the replication of those in 1 above.

A. Identify the organism(s)/agent to be used (e.g., species, strain, type, etc.):

B. Is this a Select Agent (see www.selectagents.gov/agentToxinList.htm)?

C. Does the organism contain recombinant DNA, or will recombinant DNA be constructed *in vivo* as a biologically active polynucleotide or polypeptide product? If yes, then address each of the following (if no, then N/A):

1. The source(s) of the DNA.
2. The nature of the inserted DNA sequences.
3. The host(s) and vector(s) to be used.
4. Will an attempt be made to obtain expression of a foreign gene? If so, indicate the protein that will be produced.
5. The containment conditions that will be implemented.

D. Source of the organism(s)/agent (e.g., location or name and address of lab/vendor):

E. Procedures for shipping and transportation (e.g., from facility to facility, and from room to room):

F. Location(s) where the materials are to be used and stored (include all buildings and room number and laboratories):

G. Permit information:

H. Inventory and tracking procedures (e.g., chain of custody procedures):

I. Quality control measures (e.g., procedures to prevent contamination of stocks):

Agent Hazards:

J. What particular hazards to humans, animals, and the environment are associated with these organisms/agents? (e.g., infective dose, severity of disease, mode of transmission, susceptibility to humans, stability in the environment, etc.)

Laboratory Procedure Hazards:

K. Estimated volume, amount or concentration of agents or solutions:

L. Identify known or potential sources of contamination or exposure (e.g., infected live animals, tissues, fluids, byproducts, waste, sharps, etc.)

M. Identify any procedures and equipment which could produce aerosols (e.g., pipetting, blenders, centrifuges, sonication and vortexing), and describe how the creation of aerosols and/or exposures to those aerosols will be minimized.

Biosafety, Security and Additional Precautions:

N. Biosafety Level / Risk Level (from the CDC or ABSA reference above):

O. Biosecurity Plan (the Biosecurity Plan is a description of a number of different aspects which together define the mechanisms by which biohazardous agents will be safely and securely used)

1. Physical Security: Describe procedures to prevent unauthorized access or use of the organisms/materials.

2. Biosecurity: Describe the procedures, processes, facility controls and equipment that will be used to ensure biosecurity. Include but not limited to: Description of containment; Bio-inclusion (procedures to keep biological agents in containment); Bio-exclusion (procedures to keep unwanted biological agents out of containment); Decontamination (including work surfaces, materials, cages, equipment, rooms, etc.); and Disposal procedures, including carcass disposal.

P. Specialized Risk Control Measures:

Describe specialized risk control measures to be used to protect personnel and prevent exposures. Describe items that are specific or unique for this study (e.g., personal protective equipment, immunizations or medical surveillance, training, or other specialized precautions, equipment, or practices).

T. Provide an assurance statement that all practices and procedures are in accordance with the appropriate guidelines for that biosafety/risk level of organism/materials:

U. NWRC Institutional Biosafety Committee (IBC):

Date of IBC approval letter:_____

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☐ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☐ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code
 - a) Concentration and purity:
 - b) Source:
 - c) Batch number:

For non-standard materials, describe the material/device in detail and provide the name and location of the formulation laboratory or facility that will prepare the material.

B. Describe any control or reference materials/devices

As above, for each material provide the chemical, bait or device

- 1) name or code
 - a) Concentration and purity:
 - b) Source:
 - c) Batch number:

C. Carriers, mixtures and material preparation

Give a full description of any carriers for the test/reference substance, mixing procedures, bait formulation procedures and a full description of possible contaminants and acceptable ranges for them. Include solvents, emulsifiers, dietary/bait materials and/or other materials used to dissolve or suspend the test or control substances.

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

Describe the route of administration of the test substance and give a reason for its selection.

E. Dosage

Define the dose levels of the test or control substances in appropriate units of measurement, and the frequency of administration.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

National Wildlife Research Center
Standard Operating Procedure

Title: Study Protocols	Number: AD 003.04 Effective date: 6 Dec 2010
	Replaces: AD 003.03
	Management approval: 12/6/10 [Signature] Date
Prepared by: Catherine Bens, [Signature] Laura Greiner [Signature]	Date: 12/6/10 12/13/10 Processed by QAU: [Signature] Date: 12/6/10

1.0 Purpose

To provide guidelines for the writing, review, approval and revision/change of study protocols.

- 1.1 Study is any structured scientific activity that requires committed NWRC resources such as personnel, facilities, equipment, funds.
- 1.2 Study activities requiring an approved protocol include, but are not limited to exploratory, preliminary and definitive research, formalized training, student theses and dissertations, statistical and economic analysis, operational program support activities, contracted research, or other activities requiring the use of animals, bio/hazardous materials, or specimen archiving.
- 1.3 **Studies conducted by, or under the auspices of the NWRC may not be initiated until all signatures, including that of the Director's Office, have been obtained.** Approval or exemption from IACUC or other institutional oversight still requires a fully authorized protocol, including signature from the NWRC Director's Office.

2.0 Authority

Code of Federal Regulations (CFR)

40 CFR Part 160: Good Laboratory Practice Standards (FIFRA)

40 CFR Part 792: Good Laboratory Practice Standards (TSCA)

21 CFR Part 58: Good Laboratory Practice Standards for Nonclinical Laboratory Studies (FFDCA)

9 CFR Parts 1-4: Animal Welfare Regulations

NIH Guidelines for Research Involving Recombinant DNA Molecules

3.0 Attachments

Attachment 1: Protocol and appendices templates
Attachment 2: Protocol amendment template
Attachment 3: Note to file template
Attachment 4: NWRC Protocol Decision Tree

4.0 Procedure

4.1 Protocols

Step 1: Protocol number assignment

Study protocol numbers will be assigned by the Quality Assurance Unit (QAU). Please provide the following information by email or phone call:

- Name of study director
- Protocol title
- Regulatory compliance status (non regulated, EPA GLP, FDA GLP, or other)
- NWRC Project title

Step 2: Protocol preparation and review

Prepare a study protocol in the style, content, and format of Attachment 1. Normally this is done by or under the direction of the Study Director. Delete directions provided within the protocol template as appropriate.

Distribute an electronic copy in **draft** form to the appropriate reviewers. **No signatures are required during this step.** At a minimum, the protocol must be posted to the NWRC intranet site for 2 weeks (in the *Draft Protocols* folder). To add a new document to the intranet, go to this site:

<http://animalhealth/nwrc/ga/protocolws/default.aspx>

Generally, the Study Director provides copies simultaneously to the appropriate reviewers; however this is not required. The Study Director may have as many reviewers as he/she deems necessary, but NWRC policy and the Good Laboratory Practice regulations require review by at least the following:

- **Project Leader and Assistant Director:** To ensure that the study meets requirements of the Project and to assure that adequate funding, sufficient personnel and necessary resources are available for the timely and proper conduct of the study. *Review is required by NWRC policy.*
- **Sponsor(s):** APHIS is the sponsor for the majority of the studies done by NWRC and sponsor approval is obtained with the Director's Office signature. If the sponsor is other than APHIS, have the sponsor review and approve the protocol. Sponsor approval, including the date of approval, should be obtained and documented via a signature in the

protocol, letter, facsimile, e-mail or otherwise. If a non-APHIS sponsor elects not to review the protocol, the date of approval would be the date of the contract or agreement between the sponsor and NWRC. *Sponsor approval of the protocol and any changes is required by regulation 40 CFR, Part 160, §160.120.*

- **Quality Assurance Unit (QAU):** To ensure proper format and compliance with regulations and policies, and to facilitate tracking of the protocol, study progress and final reports as per this SOP and regulation 40 CFR, Part 160, §160.35.
- **IACUC, IBC, and Individual Specialist Reviews:** Review by the Institutional Animal Care and Use Committee, Institutional Biosafety Committee, or individual specialists (e.g., analytical chemist, formulation chemist, consulting veterinarian, statistician, NEPA specialists) may be necessary as specified in the protocol format.

Step 3: Comments and Revision

Comments and suggested changes from reviewers should be addressed with appropriate changes to the protocol or other documentation. The Study Director should return revisions or responses to the reviewer to satisfy questions, comments, suggestions, or concerns about the draft protocol.

Step 4: Obtaining Approvals

Once all changes have been made, the Study Director should sign and date the finalized protocol, obtain appropriate signatures and forward the **original** hard copy and an electronic Word copy to the QAU for final processing.

The QAU will forward the protocol to the Director's Office for approval. The Director's Office will return the approved protocol to the QAU for distribution. **Approval by the Director's Office authorizes start of the study and release of the protocol for distribution to participants and use in the laboratory or field.**

The QAU will retain the **original** of the approved protocol, post a scanned copy to the NWRC intranet and provide a copy to the Study Director and the NWRC Attending Veterinarian.

Step 5: Scheduling Inspections

It is the responsibility of the Study Director to keep the QAU informed of the study schedule in a timely manner in order that QA may appropriately schedule study inspections as required by regulation. The onus is on the Study Director to keep QAU informed of study progress and status.

4.2 **Protocol Amendment/Change/Revision**

Studies will be conducted in accordance with the protocol. However, circumstances may dictate that changes are needed.

Use the *Protocol Amendment/Change/Revision form* (Attachment 2) to document changes in or revisions of an approved protocol. Document permanent, planned changes in study design, correct obvious errors in the protocol, change schedules, or resolve relatively minor, unplanned changes in study conduct.

Note: Changes involving the use of animals must be approved by IACUC and Management before that change is implemented.

Amendments/Changes/Revisions to a protocol must be signed and dated by the Study Director, Project Leader, Assistant Director, Sponsor and committees as needed, and the original forwarded to the QAU for processing, along with an electronic Word copy. The QAU will retain the original, post a scanned copy to the NWRC intranet and provide a copy to the Study Director and the NWRC Attending Veterinarian.

4.3 **Standard Operating Procedures (SOP) and SOP Deviations**

Procedures described in protocols override SOP procedures. Minor deviations from associated SOPs sometimes occur during the conduct of a study (e.g., weighing of an animal was missed one day; the time of a daily observation was not recorded). Such changes shall be authorized by the study director and documented as a Note to File (Attachment 3) or similar documentation.

United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center
PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Writing or collaborating on review papers and synthesis reports Student committee participation Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Training programs requiring the use of animals Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Collaborating on study design, data analysis, or economic analysis. Minor participation on a regulated study at the collaborating host institution A study that does not include animal use, etc.
4 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> A typical NWRC led study Major NWRC staff participation in regulated activity Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____

Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☐ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item						
Animal Use								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.</p> <p><input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix</p> <p><input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval</p> <p><input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.</p>						
Microbiological/Biohazardous Materials								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix.</p>						
Permits								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <table style="width: 100%; border: none;"> <tr> <td style="border-bottom: 1px solid black; width: 50%;"></td> <td style="border-bottom: 1px solid black; width: 20%;"></td> <td style="border-bottom: 1px solid black; width: 30%;"></td> </tr> <tr> <td style="text-align: center;">Permit(s) description</td> <td style="text-align: center;">Number</td> <td style="text-align: center;">Date</td> </tr> </table>				Permit(s) description	Number	Date
Permit(s) description	Number	Date						
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix. Contact QA/NEPA staff for ESA or eagle incidental take requirements.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.</p>						
Regulatory Standard and Test Guidelines								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study be conducted under any regulatory standard? If yes please check:</p> <p><input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i></p> <p><input type="checkbox"/> Other: _____</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____</p>						
Test, Control and Reference Material/Devices								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix. Please indicate if otherwise described in the protocol.</p>						
Historical Resources								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.</p>						
Material Transfer Agreement								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement.</p>						
Analytical Chemistry								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?</p> <p>If yes, attach Analytical Chemistry Appendix.</p>						

PART THREE: DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
 - ☐ *Manuscript/review article collaboration*
 - ☐ *Training program requiring the use of animals*
 - ☐ *Data analysis, interpretation and reporting*
 - ☐ *Other: _____*

Collaboration:	Name	Address or Organization	Role in Project

Start Date:

End Date:

Archive Date:

- Anticipated Project Outcome:
- ☐ Manuscript
 - ☐ Report
 - ☐ Other: _____

Materials to be archived to close this activity:

Description of Project and NWRC Activities and Participation:

Comments:

Attachments:
(e.g. Material Transfer Form, IACUC approval, etc.)

PART FOUR: FULL NWRC STUDY PROTOCOL**1. Key Personnel**

Name	Organization	Role in Study
Study Director		
Other Investigators, Collaborators, Cooperators, and Consultants		

2. Testing Facilities

Name	Address	Role in Study

3. Sponsor

Name	Address	Contract No.

4. Schedule

Proposed Experimental Start Date:
Proposed Experimental Termination Date:
Proposed Study Completion/Archive Date:

5. Background and Justification

Give the rationale for the study with an analysis of the problem situation and a clear statement of need and justification. Include a summary of the literature reviewed.

6. Related Protocols

List by Protocol Number

7. Assurance of Non-Duplication of Studies

Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.

8. Objective/Hypotheses

Give concise statements as to the objective of the study and the hypotheses to be tested.

9. Methods/Procedures

Give a logical sequence of events leading toward attainment of the objectives including the type and frequency of tests, measurements, and analyses to be made. The level of detail should be at a level which would allow an independent third party or educated lay person to read and conceptually understand it and a scientific researcher to conduct or repeat the study based solely on the protocol. For field studies include a description of the field sites where the study will be conducted. Refer to details in the attached appendices as appropriate. Analytical chemistry procedures may be indicated in the attached appendices, but all other methods and procedures must be provided directly or by reference to the appropriate SOP(s). Information frequently forgotten includes randomization schemes and procedures, bioanalytical assays, and a comprehensive description of all procedures and methods (field and lab), etc.

10. Experimental Design and Statistical Analyses

Describe the experimental design including methods for control of bias. Include sample sizes, sketches, and narrative as needed to make the design clear. Give a statement of the proposed statistical method or methods to be used. If a statistician was consulted for assistance in study design, give the date of the consultation and the name and affiliation of the person consulted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a.
 - b.
 - c.
 - d.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx
A. Salary and Benefits			
B. Facilities (in addition to existing facility or space costs)			
C. Equipment			
D. Supplies			
E. Animal Care Costs			
F. Operating Costs (travel, misc. services, etc)			
TOTAL	\$0	\$0	\$0

14. Human Health and Safety

Cite the appropriate SOP(s) or explain briefly the safety precautions, equipment, and procedures to be used for potentially hazardous conditions. State whether or not the proposed research has any potential for risk to the health or safety to members of the public, and, if so, explain how such risk(s) will be minimized or avoided.

15. Staff Qualifications

[Standard text revise as needed] All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study.

16. Archiving

[Standard text revise as needed] All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

[Standard text revise as needed] Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistance Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

List in alphabetical order by author.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☐ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☐ NEPA and ESA Appendix
 - ☐ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: **(include appropriate title)** _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal Description

1) Animals:

Species, subspecies (if applicable):

Breed, strain and substrain (if applicable):

Total Number and Sex:

Body weight range:

Age:

B. Rationale for involving animals, for appropriateness of species, and for numbers Provide justification why this study requires the use of animals, and for the numbers to be used.

1) Rationale for involving animals:

2) Rationale for appropriateness of the species to be used:

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):

C. Source

Describe where the animals will be trapped or obtained, or identify the vendor by name and address.

D. Method of identification of animals

Cite the appropriate SOP(s) or explain briefly how animals will be marked or identified to prevent misidentification.

E. Trapping/Collecting

Cite the appropriate SOP(s) or explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.)

F. Transport

Cite the appropriate SOP or explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, etc.

G. Handling/restraint

Cite the appropriate SOP(s) or explain briefly how the animals will be held or restrained (manual vs. chemical) throughout study.

H. Quarantine

Cite the appropriate SOP, or describe the procedure for the quarantine of animals.

I. Housing/maintenance

Cite the appropriate SOP(s) or explain briefly how housing/maintenance will be done (including information on feeder animals if used).

J. Dietary contaminant exposure

Are there any contaminants or diet supplements that are reasonably expected to be present in the dietary materials, drinking water, or bedding material and are known to be capable of interfering with the purpose or conduct of the study? If so, please describe control/testing mechanism.

K. Disposition of animals

Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study, or cite the appropriate SOP(s).

L. Animal pain or distress**1) Consultation with Attending Veterinarian:**

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: _____

Date of Consultation: _____

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☐ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

M. Euthanasia

Describe the appropriate method of euthanasia to be used (cite the appropriate SOP or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)

N. IACUC Approval

Date of IACUC Approval Letter: _____

O. Staff Qualifications

List the study participants that will be working independently with animals and provide their qualifications/certifications (i.e. name, title, and a brief description of training/experience).

Analytical Chemistry Appendix

If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

- A. Number of samples to be analyzed (by type):**
- B. Storage conditions (temperature, container type, light/dark, duration):**
- C. Method title and number:**
- D. ACP Leader approval: _____ Date: _____**
(attach email or letter of concurrence from Analytical Services Project Team Leader)

If chemical analysis will be made by a laboratory outside of NWRC, include A-C above and attach the method to be used.

Column E Explanation

1. Registration Number: 84-F-0001
2. Number of animals used in this study during this reporting period:
3. Species (common name) of animals used in study during this reporting period:
4. Explain procedure producing pain and/or distress:
5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):
6. What, if any, federal regulations require this procedure?

Agency:

CFR:

Material Transfer Agreement

STANDARD AGREEMENT
U. S. Department of Agriculture
Animal and Plant Health Inspection Service / Wildlife Services
National Wildlife Research Center

PARTIES:

APHIS: USDA, APHIS
 National Wildlife Research Center
 Scientist Address
 City, State Zip
 Tel: Telephone # of Scientist
 FAX: FAX # of Scientist
 E-Mail: E-mail address of Scientist

Recipient: Company Name
 Company Address
 City, State Zip of Company
 Tel: Telephone # of Recipient
 FAX: FAX # of Recipient
 E-mail: E-mail address of Recipient

PURPOSE:

To provide Recipient with [redacted] and associated know how, hereinafter collectively referred to as the Material.

The Material is released to Recipient under the following conditions:

1. The Material and associated know-how shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall remain the property of APHIS and shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material and shall provide APHIS with any manuscript that describes the work with the Material prior to submission for publication and acknowledge APHIS' contribution to the work reported.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health and Inspection Service, the Center for Disease Control, and /or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.

8. Upon completion of the activities performed using the Material, the Material shall be returned, destroyed or otherwise disposed of as instructed by APHIS.
9. Recipient shall meet with U.S. Department of Agriculture representatives to determine inventorship if an invention should arise from work with the Material.
10. Recipient shall not disclose Material marked "Confidential" or "Proprietary" to any third party without written permission from APHIS.
11. Material shall be excluded from the confidentiality requirements of this Agreement if: (1) Recipient had possession of the Material prior to disclosure; (2) the Material is generally available to the public at the time of disclosure; (3) the information becomes generally available to the public through no fault of Recipient after disclosure; or (4) after disclosure, Recipient receives the Material from a third party having the right to the Material and who does not impose a confidentiality obligation upon Recipient.
12. If the parties hereto decide, at some future date, to engage in a cooperative research project or program using the Material, a formal Cooperative Research and Development Agreement, or other research Agreement, must be negotiated and entered into between the parties. Such an Agreement shall supersede this Material Transfer Agreement.
13. This Material Transfer Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

This Material Transfer Agreement shall become effective upon date of final signature and shall continue in effect for a period of [state a period of one to five (1-5) years].

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:

QA#: Permit Information
(Type and Number):

Typed name/Title

Signature (NWRC APHIS Scientist)

Date

Typed Name/Title

Signature (NWRC APHIS Assistant
Director)

Date

ACCEPTED FOR RECIPIENT:

Typed Name/Title

Signature

Date

APPROVED:

Typed Name/Title

Signature (Technology Transfer
Coordinator)

Date

Original: NWRC Agreements Specialist

cc: Technology Transfer Program Manager, Quality Assurance Unit

Material Transfer Agreement

ANIMAL / ANIMAL TISSUE TRANSFER AGREEMENT
U. S. Department of Agriculture
Animal and Plant Health Inspection Service / Wildlife Services
National Wildlife Research Center

PARTIES:

APHIS: USDA, APHIS
National Wildlife Research Center
Scientist Address
City, State Zip
Tel: Telephone # of Scientist
FAX: FAX # of Scientist
E-Mail: E-mail address of Scientist

Recipient: Company Name
Company Address
City, State Zip of Company
Tel: Telephone # of Recipient
FAX: FAX # of Recipient
E-mail: E-mail address of Recipient

PURPOSE:

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

[Table may be adjusted as needed]

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS' informed of the results obtained through your use of the Material and shall provide APHIS with any manuscript that describes the work with the Material and acknowledge APHIS' contribution to the work reported when appropriate.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result

5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals. biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.
7. Upon completion of the activities performed using the Material, the Material shall be [redacted] *[for example, returned to ..., destroyed by..., disposed of as instructed by APHIS].*
8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated]*THIS MATERIAL MAY BE INFECTED WITH PATHOGENS *[be specific when warranted]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.
9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.
10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed.

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

QA#:	Permit Information (Type and Number):
------	--

Typed name/Title

Signature (NWRC APHIS Scientist)

Date _____

Typed Name/Title

Signature (NWRC APHIS Project Leader)

Date _____

ACCEPTED FOR RECIPIENT:

Typed Name/Title

Signature
(Technology Transfer Coordinator)

Date _____

Original: Quality Assurance Unit

Microbiological/Biohazardous Materials Use Appendix

NWRC proposed research or testing activities which involve the use of microbiological organisms or biohazardous agents at or above a Biosafety Level 2 or Risk Level 2, or use recombinant DNA *in vivo*, require this appendix to be completed and submitted to the NWRC IBC for review and approval.

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at www.cdc.gov/od/ohs/biosfty/biosfty.htm for the definitions and lists of BioSafety Level 2 organisms and above.

Reference the American Biological Safety Association's (ABSA) "Risk Group Classification for Infectious Agents" at <http://www.absa.org/resriskgroup.html> for the definitions and lists of Risk Level 2 agents and above.

Reference the National Institute of Health's (NIH) Guidelines for Recombinant DNA and Gene Transfer at www4.od.nih.gov/oba/rac/documents1.htm for specific practices for constructing and handling recombinant DNA and organisms/viruses containing recombinant DNA molecules. Definition of recombinant DNA; 1) Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or 2) Molecules that result from the replication of those in 1 above.

A. Identify the organism(s)/agent to be used (e.g., species, strain, type, etc.):

B. Is this a Select Agent (see www.selectagents.gov/agentToxinList.htm)?

C. Does the organism contain recombinant DNA, or will recombinant DNA be constructed *in vivo* as a biologically active polynucleotide or polypeptide product? If yes, then address each of the following (if no, then N/A):

1. The source(s) of the DNA.
2. The nature of the inserted DNA sequences.
3. The host(s) and vector(s) to be used.
4. Will an attempt be made to obtain expression of a foreign gene? If so, indicate the protein that will be produced.
5. The containment conditions that will be implemented.

D. Source of the organism(s)/agent (e.g., location or name and address of lab/vendor):

E. Procedures for shipping and transportation (e.g., from facility to facility, and from room to room):

F. Location(s) where the materials are to be used and stored (include all buildings and room number and laboratories):

G. Permit information:

H. Inventory and tracking procedures (e.g., chain of custody procedures):

I. Quality control measures (e.g., procedures to prevent contamination of stocks):

Agent Hazards:

J. What particular hazards to humans, animals, and the environment are associated with these organisms/agents? (e.g., infective dose, severity of disease, mode of transmission, susceptibility to humans, stability in the environment, etc.)

Laboratory Procedure Hazards:

K. Estimated volume, amount or concentration of agents or solutions:

L. Identify known or potential sources of contamination or exposure (e.g., infected live animals, tissues, fluids, byproducts, waste, sharps, etc.)

M. Identify any procedures and equipment which could produce aerosols (e.g., pipetting, blenders, centrifuges, sonication and vortexing), and describe how the creation of aerosols and/or exposures to those aerosols will be minimized.

Biosafety, Security and Additional Precautions:

N. Biosafety Level / Risk Level (from the CDC or ABSA reference above):

O. Biosecurity Plan (the Biosecurity Plan is a description of a number of different aspects which together define the mechanisms by which biohazardous agents will be safely and securely used)

1. Physical Security: Describe procedures to prevent unauthorized access or use of the organisms/materials.

2. Biosecurity: Describe the procedures, processes, facility controls and equipment that will be used to ensure biosecurity. Include but not limited to: Description of containment; Bio-inclusion (procedures to keep biological agents in containment); Bio-exclusion (procedures to keep unwanted biological agents out of containment); Decontamination (including work surfaces, materials, cages, equipment, rooms, etc.); and Disposal procedures, including carcass disposal.

P. Specialized Risk Control Measures:

Describe specialized risk control measures to be used to protect personnel and prevent exposures. Describe items that are specific or unique for this study (e.g., personal protective equipment, immunizations or medical surveillance, training, or other specialized precautions, equipment, or practices).

T. Provide an assurance statement that all practices and procedures are in accordance with the appropriate guidelines for that biosafety/risk level of organism/materials:

U. NWRC Institutional Biosafety Committee (IBC):

Date of IBC approval letter: _____

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☐ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☐ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

Consultations:

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code
 - a) Concentration and purity:
 - b) Source:
 - c) Batch number:

For non-standard materials, describe the material/device in detail and provide the name and location of the formulation laboratory or facility that will prepare the material.

B. Describe any control or reference materials/devices

As above, for each material provide the chemical, bait or device

- 1) name or code
 - a) Concentration and purity:
 - b) Source:
 - c) Batch number:

C. Carriers, mixtures and material preparation

Give a full description of any carriers for the test/reference substance, mixing procedures, bait formulation procedures and a full description of possible contaminants and acceptable ranges for them. Include solvents, emulsifiers, dietary/bait materials and/or other materials used to dissolve or suspend the test or control substances.

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

Describe the route of administration of the test substance and give a reason for its selection.

E. Dosage

Define the dose levels of the test or control substances in appropriate units of measurement, and the frequency of administration.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

Study Director: _____ Amendment No.: _____ Page ____ of ____

Study title: _____

Changes in schedule:

<input type="checkbox"/>	No schedule changes		
<input type="checkbox"/>	Experiment Start Date:	(current) _____	(revised) _____
<input type="checkbox"/>	Experiment Termination Date:	(current) _____	(revised) _____
<input type="checkbox"/>	Study Completion/Archive Date:	(current) _____	(revised) _____

Protocol section/subsection/appendix to be changed:**Description of revisions:** *(Please provide the level of detail normally required in the protocol)***Justification/reason(s) for changes and impact on study:** *(If dates are changed, please provide a description of current status of study and remaining study plan/schedule.)*

Study Director: _____ Date _____

Project Leader: _____ Date _____

Assistant Director: _____ Date _____

NWRC IACUC / IBC (as needed): _____ Date _____

QAU received: _____ QAU reviewed: _____

Note: Sponsor approval is needed for all non-NWRC sponsored research

United States
Department of
Agriculture



Animal and
Plant Health
Inspection
Service

Wildlife Services

National Wildlife
Research Center

Date:

Subject: Note to file for QA-_____

To:

Copies to:

Signature

Date



AD003.04

Safeguarding American Agriculture

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NWRC Protocol Decision Tree

Are NWRC staff involved in study design, data collection, experiments, or animal studies?

NO

Activities do not involve regulated research activities* and are generally limited to NWRC personnel time

Classification 1

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 3 (Description of Activities)

EXAMPLES:

- Writing or collaborating on review papers and synthesis reports
- Student committee participation
- Analyzing or writing up data collected under operational or other contexts without prior input from NWRC scientists

YES

Activities involve regulated research activities*

Classification 2

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 3 (Description of Activities)
- ✓ NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval, as applicable.
- ✓ NWRC Material Transfer Agreement (Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable)

EXAMPLES:

- Training programs requiring the use of animals
- Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)
- Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)

NWRC activities do not involve regulated research activities*.

Classification 3

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 4 (full NWRC Study Protocol)
- ✓ Collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.

EXAMPLES:

- Collaborating on study design, data analysis, or economic analysis.
- Minor participation on a regulated study at the collaborating host institution
- A study that does not include animal use, etc.

NWRC activities involve regulated research activities*.

Classification 4

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 2 (Regulatory Considerations)
- ✓ Part 4 (full NWRC Study Protocol)
- ✓ Required documents under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.

EXAMPLES:

- A typical NWRC led study
- Major NWRC staff participation in regulated activity
- Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use"

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#)
Subject: RE: GonaCon Bison testing
Date: Friday, November 29, 2013 9:04:00 AM

I thought those dates might interfere with the STAS annual retreat to St. Thomas. But that's not until the following week.

So I should be able to make it up to Montana Jan 6. Perhaps we can convince the pilot to let us pop in on the STAS time share in Jackson Hole when we are done.

Hope you all had a great Thanksgiving!

Pauline

From: Rhyan, Jack C - APHIS
Sent: Tuesday, November 26, 2013 4:23 PM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: RE: GonaCon Bison testing

Hmmm....Maybe B is for Best and A is for.....well, never mind. The bison must be piling up at Hell Roaring or Gardiner. Wow! We will confab on the dates and get back to you.

I must leave soon as tomorrow afternoon, they have actually given the STAS elite administrative leave. I must get home and prepare my luggage so that when the Leer arrives tomorrow to take us to LaGuardia from where we will be helo'ed to the South Fork to get our ponies ready, I will be ready. (Sheesh! I didn't know the word had got out.)

Eucerin will help those knuckles.

Jack

From: Clarke, Patrick R. - APHIS
Sent: Tuesday, November 26, 2013 3:24 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: GonaCon Bison testing

Becky and I are looking at January 7-9, 2014 (Tuesday, Wednesday, Thursday) to work the GonaCon bison up here at Corwin Springs. I'm not sure if we'll need all three days, but certainly two. YNP has indicated they may be trapping and sending animals to slaughter as early as December. So the other factor in the mix this particular week is if the YNP trap is working we can also be getting "sniffer" samples and GonaCon cohort #2 animals.

Do these days work for youthe A Team.....???

I speak of you as the A Team now that they have split us apart and you have joined the special company of technical analysts, geniuses, savants, etc. in STAS....while Becky and I are relegated to the knuckle-draggers of District 5 in SPRS. I heard STAS people get every other Friday off as admin leave to either 1) reorganize their wine cellars or 2) play polo in the Hamptons. Is this true???

Have a good Thanksgiving,

The B Team

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: [Frey, Rebecca K \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Clarke, Patrick R. \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [Rick Wallen](#)
Subject: Re: GonaCon call?
Date: Tuesday, May 24, 2011 1:09:31 PM

Thursday or friday will work for me or tuesday PM.
Becky Frey

From: Matt McCollum
Sent: 05/24/2011 04:48 PM GMT
To: Jack Rhyan; Jason Lombard; "Jenny_Powers@nps.gov" <Jenny_Powers@nps.gov>; "Margaret_Wild@nps.gov" <Margaret_Wild@nps.gov>; Patrick Clarke; Pauline Nol; Rebecca Frey; "Rick_Wallen@nps.gov" <Rick_Wallen@nps.gov>
Subject: GonaCon call?

Hi there,

It'd be good to get another call together to talk about where we are at with this. I've attached the final ACUC protocol that has been submitted.

Thursday the 26th is wide open for me at this point. Friday afternoon would work, or pretty much anytime next week. Let me know your availability please.

Thanks,
Matt

From: [Nol, Pauline \(APHIS\)](#)
To: [Frey, Rebecca K \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Clarke, Patrick R. \(APHIS\)](#); [Rick Wallen](#)
Subject: RE: GonaCon call?
Date: Tuesday, May 24, 2011 1:48:00 PM

Sorry, I need to revise my last reply-I'm available Thursday and Friday afternoons and should be around most of next week. Pauline

From: Frey, Rebecca K (APHIS)
Sent: Tuesday, May 24, 2011 1:09 PM
To: McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); Jenny_Powers@nps.gov; Margaret_Wild@nps.gov; Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Rick Wallen
Subject: Re: GonaCon call?

Thursday or Friday will work for me or Tuesday PM.
Becky Frey

From: Matt McCollum
Sent: 05/24/2011 04:48 PM GMT
To: Jack Rhyan; Jason Lombard; "Jenny_Powers@nps.gov" <Jenny_Powers@nps.gov>; "Margaret_Wild@nps.gov" <Margaret_Wild@nps.gov>; Patrick Clarke; Pauline Nol; Rebecca Frey; "Rick_Wallen@nps.gov" <Rick_Wallen@nps.gov>
Subject: GonaCon call?

Hi there,

It'd be good to get another call together to talk about where we are at with this. I've attached the final ACUC protocol that has been submitted.

Thursday the 26th is wide open for me at this point. Friday afternoon would work, or pretty much anytime next week. Let me know your availability please.

Thanks,
Matt

From: [McCollum, Matthew P \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Rhyen, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Clarke, Patrick R. \(APHIS\)](#); [Rick Wallen](#)
Subject: RE: GonaCon call?
Date: Wednesday, May 25, 2011 3:15:10 PM

So it sounds like everyone I've heard back from is available next Tuesday May 31 at 2:00 in the afternoon.

I'll go ahead and set up a call for then unless I hear otherwise.

I'll send out the call in numbers tomorrow.

Thanks,
Matt

From: Nol, Pauline (APHIS)
Sent: Tuesday, May 24, 2011 1:48 PM
To: Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS); Rhyen, Jack C (APHIS); Lombard, Jason E (APHIS); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); Clarke, Patrick R. (APHIS); Rick Wallen
Subject: RE: GonaCon call?

Sorry, I need to revise my last reply-I'm available Thursday and Friday afternoons and should be around most of next week. Pauline

From: Frey, Rebecca K (APHIS)
Sent: Tuesday, May 24, 2011 1:09 PM
To: McCollum, Matthew P (APHIS); Rhyen, Jack C (APHIS); Lombard, Jason E (APHIS); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Rick Wallen
Subject: Re: GonaCon call?

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Becky Frey

From: Matt McCollum
Sent: 05/24/2011 04:48 PM GMT
To: Jack Rhyen; Jason Lombard; "Jenny_Powers@nps.gov" <[Jenny_Powers@nps.gov](#)>; "Margaret_Wild@nps.gov" <[Margaret_Wild@nps.gov](#)>; Patrick Clarke; Pauline Nol; Rebecca Frey; "Rick_Wallen@nps.gov" <[Rick_Wallen@nps.gov](#)>
Subject: GonaCon call?

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Thanks,
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From: [Nol, Pauline \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); "[Jenny_Powers@nps.gov](#)"; "[Margaret_Wild@nps.gov](#)"; [Clarke, Patrick R. \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); "[Rick_Wallen@nps.gov](#)"
Subject: Re: GonaCon call?
Date: Tuesday, May 24, 2011 1:24:12 PM

Both those times will work for me, and most of next week as well.

Pauline

From: McCollum, Matthew P (APHIS)
Sent: Tuesday, May 24, 2011 11:48 AM
To: Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); Jenny_Powers@nps.gov <Jenny_Powers@nps.gov>; Margaret_Wild@nps.gov <Margaret_Wild@nps.gov>; Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov <Rick_Wallen@nps.gov>

Subject: GonaCon call?

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Thursday the 26th is wide open for me at this point. Friday afternoon would work, or pretty much anytime next week. Let me know your availability please.

Thanks,
Matt

From: [Rhyan, Jack C \(APHIS\)](#)
To: [Fagerstone, Kathleen A \(APHIS\)](#)
Cc: [Nol, Pauline \(APHIS\)](#); [Miller, Lowell A \(APHIS\)](#)
Subject: RE: GonaCon Conference Call
Date: Tuesday, June 21, 2011 11:23:58 AM

Sure, I put our names on the Products conf room (Mt Princeton) See you all at 1:30 today. Kathy, please let John know if you think he needs to be there.

Jack

From: Fagerstone, Kathleen A (APHIS)
Sent: Tuesday, June 21, 2011 10:55 AM
To: Rhyan, Jack C (APHIS)
Subject: RE: GonaCon Conference Call

Jack—Do we want to all call from one phone?
Kathy

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, June 21, 2011 10:08 AM
To: Nol, Pauline (APHIS); Fagerstone, Kathleen A (APHIS); Miller, Lowell A (APHIS)
Subject: FW: GonaCon Conference Call

FYI

From: Stephens, Stephanie H (APHIS)
Sent: Monday, June 20, 2011 2:21 PM
To: Donch, Debra A (APHIS); Willard, Tracy A (APHIS); Edmundson, Jack P (APHIS); Rhyan, Jack C (APHIS); Gutierrez, Vicki L (APHIS); Nasr, Ann M (APHIS)
Subject: GonaCon Conference Call

Hi Everyone-

Based on responses about availability, I've reserved a conference call line tomorrow for us to discuss the questions below on the GonaCon bison protocol. Here are the meeting details:

Date: Tuesday, June 21, 2011
Time: 3:30 ET (1:30 MT)
Phone: (b) (6)
Code: (b) (6)

Jack R., I can pass this information along to Kathy Fagerstone if you think it would be good to have her participation on the call as well to weigh in on APHIS Wildlife Services issues related to this project.

Thanks,

Stephanie

Stephanie Stephens
USDA APHIS PPD
Environmental and Risk Analysis Services
Headquarters: 4700 River Road, Unit 149, Riverdale, MD 20737
Utah Office phone/fax: (435) 658-5134

From: Edmundson, Jack P (APHIS)
Sent: Friday, June 10, 2011 12:59 PM
To: Rhyan, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: Some Q's on the GonaCon protocol and request for conf call

Hi, Jack. We pulled the Bison Team together the other day to begin work in earnest on the GonaCon EA. The first thing we did was go through the protocol with a fine-toothed comb to be sure we understood exactly what we are planning to do. Based on some things we have seen from BFC we suspect that they will be all over the study and watching like a hawk. As I understand it, the protocol you sent us is the final one that has been approved by NPS and a permit has been issued based on it. (In other words, APHIS shouldn't change anything in it because it would be a major paperwork hassle.) With that as background, we do have a few comments/questions about the protocol:

- How come we need a YNP permit to do work outside of the Park? And what exactly does the permit cover and not cover?
- For NEPA purposes, is the lead agency APHIS or APHIS-VS? Will NPS (or NPS and APHIS-WS) officially be a cooperator in the EA? If NPS is an official cooperator, it could add additional review/approval time because NPS would have to be involved. Does NPS expect to be a NEPA Cooperator?
- What is the relationship of the study to FIFRA Registration?
- What are the roles of WS and NPS? Will they actually help in the field? Analyze info? Review/comment on things?
- The study says it starts on June 1, 2011, presumably because we collected animals after that? From a NEPA standpoint, we would prefer to have it start in 2012 when we begin to inject animals. We have already said that NEPA did not need to be done to collect animals for research. And, if we say it has already started, then technically NEPA should already be completed. (Also, for a 7 year study, it should end in 2019, not 2017.)
- Is Cammie Johnson our statistician? Should we list her in the investigators?
- The 3rd Objective does not seem to have a hypothesis associated with it. Also, the only thing in the Methods/Procedures section that could relate is the paragraph talking about what is to happen if there is an abortion in the field. It is not tied together very clearly (at least not enough for us to explain it to the public, as we must do in the EA).
- In several places we talk about marking animals, but it is not real clear how. For instance on p.4 #8 we mention collars, but elsewhere we talk about ear tags and microchips. We will need to talk about which methods we use and when.
- There is some confusion in our minds about the months when things happen. For instance,

on page 5 we identify a time period when bulls will be separated from cows as outside the breeding season (from Oct to July), and the abortion/calving season from Feb to Aug. These dates will allow bulls to be with cows in August, when they could be exposed to abortions/birth-related shedding.

- We were confused by the statistics section and will probably need to be walked through that so that we can understand what we are measuring and what it means.
- There is also some confusion about when we can donate to food banks, when incineration will be used, when chemicals will be used for immobilization and/or euthanasia.

There are additional small points we would want to just talk with you about to get them straight in our minds or to ask your advice as to how to best present them in an EA. Can we organize a conference call with you to talk some of these things out? Since I am getting ready to retire, I'll be phasing out of the bison business (one of my regrets at retiring) and Stephanie Stephens will be taking my place. Since she (and Vicki) will be leading the NEPA effort, she will be getting in contact with you to set up the conference call, but we wanted you to have at least a partial list of the things we have been thinking about.

Jack E

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: RE: GonaCon Data Jan 8-9 2013----Vaginal Transmitter Frequencies Jan 25 2013
Date: Friday, January 25, 2013 3:31:00 PM

Well done sir! Hopefully the missing one didn't come out with a fetus, get eaten, and then transported away!

Have a great weekend!

From: Clarke, Patrick R. - APHIS
Sent: Friday, January 25, 2013 3:06 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS
Subject: GonaCon Data Jan 8-9 2013----Vaginal Transmitter Frequencies Jan 25 2013

I can pick up a signal from all but one of our 22 vaginal transmitters!

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA-APHIS-VS-WR

406-388-5162

From: [Orahood, Darcy S - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Eckery, Douglas C - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: Gonacon meeting?
Date: Wednesday, May 14, 2014 11:07:14 AM

Thanks for initiating this Matt. I think it will be really helpful to discuss what GonaCon experiments have been done, compare formulations and results, discuss concerns about the current vaccine, etc. Doug will be out of the office May 21-23, May 28-30, and June 4-6. So we'll have to look at Monday and Tuesday options over the next three weeks or hold off until the week of June 9th.

This could be tough with so many busy schedules so let's do it this way – please select the dates/times you are available on the following Doodle poll: <http://doodle.com/9y9csk8yh7apzwy3>

Darcy Orahood

Biological Science Technician

National Wildlife Research Center

4101 LaPorte Avenue

Fort Collins, CO 80521

Phone (970) 266-6061

From: Rhyan, Jack C - APHIS
Sent: Wednesday, May 14, 2014 10:49 AM
To: McCollum, Matthew P - APHIS; Eckery, Douglas C - APHIS
Cc: Orahood, Darcy S - APHIS; Nol, Pauline - APHIS
Subject: RE: Gonacon meeting?

All us'ins on the email should be there! I'm here until the week of Memorial Day (May 26th).

Jack

From: McCollum, Matthew P - APHIS
Sent: Wednesday, May 14, 2014 10:29 AM
To: Rhyan, Jack C - APHIS; Eckery, Douglas C - APHIS
Cc: Orahood, Darcy S - APHIS; Nol, Pauline - APHIS
Subject: Gonacon meeting?

Hi,

Darcy and I were just chatting in the hallway and it seems to me that a pow-wow (pow-ow?) might be in order to talk about Gonacon. What has been done and what can be done in future. Any interest? If so, who all should be there?

I'm pretty free next week except for Friday.

Thanks,

Matt McCollum

Wildlife Disease Biologist

USDA/APHIS/VS

Wildlife/Livestock Disease Investigations Team

4101 Laporte Ave

Fort Collins, CO 80521

(970)266-6233 Office

(b) (6) Mobile

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Eckery, Douglas C - APHIS](#)
Cc: [Orahood, Darcy S - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: Gonacon meeting?
Date: Wednesday, May 14, 2014 10:49:15 AM

All us'ins on the email should be there! I'm here until the week of Memorial Day (May 26th).

Jack

From: McCollum, Matthew P - APHIS
Sent: Wednesday, May 14, 2014 10:29 AM
To: Rhyan, Jack C - APHIS; Eckery, Douglas C - APHIS
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From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Eckery, Douglas C - APHIS](#)
Cc: [Orahood, Darcy S - APHIS](#)
Subject: RE: Gonacon meeting?
Date: Wednesday, May 14, 2014 10:46:00 AM

Good idea!

I'm not very available next week except for Friday. So can we shoot for the week after?

P

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: McCollum, Matthew P - APHIS
Sent: Wednesday, May 14, 2014 10:29 AM
To: Rhyan, Jack C - APHIS; Eckery, Douglas C - APHIS
Cc: Orahood, Darcy S - APHIS; Nol, Pauline - APHIS
Subject: Gonacon meeting?

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To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Eckery, Douglas C - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: Gonacon meeting?
Date: Monday, May 19, 2014 8:22:21 AM

Good morning everyone,

It looks like **Monday, June 2nd at 2:30 PM** works for all of us to meet and discuss the bison GonaCon work. I reserved the Mt. Evans conference room for us... so see you there!

Thanks,

Darcy Orahood

Biological Science Technician

National Wildlife Research Center

4101 LaPorte Avenue

Fort Collins, CO 80521

Phone (970) 266-6061

From: McCollum, Matthew P - APHIS
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From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: GonaCon Shopping List
Date: Tuesday, February 07, 2012 9:08:00 AM

Hiya, yeah, I'll bet Biomark would have some ideas. They use multiple sensing pit tags in bats too, etc.

We do need to keep in mind that a vag transmitter might come out in a different place from the fetus and the placenta. So we will likely have to hand tag the calf or associated pieces and parts.

From: McCollum, Matthew P - APHIS
Sent: Tuesday, February 07, 2012 9:03 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

Good questions... Being in the "big idea" business, I really don't know much more yet. My thought is that there is technology out there that might work- gopher burrow sensors designed to record animals passing by them might be used for instance. I'm not sure if the tags would work as they are in the animals or if we'd have to put some in on the tip of their nose. I'd think we could incorporate a pit tag into a vag transmitter.

Biomark is one company that handles RIFD implants, they might be helpful in putting something together.

Who's afraid of the big bad wolf? Ha ha ha ha haaa...

Matt

From: Frey, Rebecca K - APHIS
Sent: Tuesday, February 07, 2012 8:52 AM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

I guess I am not up to speed on PIT tags that record contact. Is that just one recorder on the fetus and any type of RFID tag in the "contact" animals or is it a complete system. Remember we have already tagged these animals with RFID tags.

We can use the security cameras(Jack and I are working that out with Karin etc.), and we will be putting in VIT's to know when births take place. Is it possible to have this recording PIT inserted into the VIT? So it all comes out together and we don't miss any data?

Becky

And if anyone has anymore acronym ideas, lets have them. I am tired of actually typing whole words for anything ☺

Rebecca Frey
Wildlife Biologist/Disease Specialist
Greater Yellowstone Area
406-333-4425

From: McCollum, Matthew P - APHIS
Sent: Friday, February 03, 2012 4:18 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

So Jack and I did a little brain storming about proximity data and how to collect it. The fear is that if we use proximity logger collars, we'll be collecting data that doesn't mean anything. Jack came up with PIT tags and readers. We could put a reader on, under, or near an aborted fetus or birth-site and use it to record "contact" with PIT tagged animals. Also, putting up cameras to record behavior. (can we use the security cameras for anything?)

Matt

From: Rhyan, Jack C - APHIS

Sent: Friday, February 03, 2012 2:38 PM

To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: RE: GonaCon Shopping List

Becky,

I agree. I'd rather not use the 2 that may have had a transient titer. I think we should ask for 10K for drugs. Proximity collars. Thanks for reminding us. We don't especially need them until our first calving season do we? But we should be trying to get them.

Jack

From: Frey, Rebecca K - APHIS

Sent: Friday, February 03, 2012 11:33 AM

To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: RE: GonaCon Shopping List

I have a question or two to consider for all of you.

I gave Rick our shopping list, though there is some discrepancy in the numbers of positive and negative animals. I think we should proceed as if the 2 animals that are unknowns, will be "extras" and we worry about them later. These 2 "unknowns" were called positive at the trap, then were not tested by us again until December, and tested negative in December. I pulled the serum from YNP from the original test, and DOL called them both positive. We will of course retest these two, but I don't want to do it without the need to get the whole bunch in. Does that sound good?

Here is the shopping list I gave Rick. I did not include the two animals above in our totals which should give 2 extra animals if we get the maximum listed below for each category.

After crunching the numbers including our latest test results, our 1st priority is negative bulls, 1-3 years old preferred, but we may be less picky here We will need a minimum of 8, and hopefully a max of 16 (if seroconversion is about 50%) to accommodate all of the test groups.

2nd priority: We need 4 positive females (1-3 yo's) to start the 1st 2 test groups.

Then: 32-40 seropositive 1-3 yo's non-pregnant

3-5 seronegative 1-3 yo's non-pregnant

On another note, we need either GPS collars or proximity monitors for the study. Did we include that in the budget? We need to decide what is the best technology to tell us what we need to know. I am getting collars back from Rick as well as some VIT's. Will let you know how many but I think it is roughly 20. IF we get all of the animals we need this year, we will potentially have 96 females that will require darting after calving. At Jack's estimate of \$100/animal, we are looking at a minimum or \$9600/yr for drugs. If we only get enough to do 2 pens this year, we are looking at \$4800 minimum, then going up as we get more animals. I think we just need to include \$10,000/year.

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

Greater Yellowstone Area

406-333-4425

From: Clarke, Patrick R. - APHIS

Sent: Thursday, February 02, 2012 4:25 PM

To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: GonaCon Shopping List

YNP says if the bison movement is right they are prepared to trap as early as Feb 13th.....the first animals would go to the GonaCon study. Do we have a "shopping list" of the animals we still need for the study (age ,sex, sero-status, etc.) that we can share with YNP?

Cheers,

P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: [McCollum, Matthew P - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon Shopping List
Date: Tuesday, February 07, 2012 9:02:39 AM

Good questions... Being in the "big idea" business, I really don't know much more yet. My thought is that there is technology out there that might work- gopher burrow sensors designed to record animals passing by them might be used for instance. I'm not sure if the tags would work as they are in the animals or if we'd have to put some in on the tip of their nose. I'd think we could incorporate a pit tag into a vag transmitter.

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Jack

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Sent: Friday, February 03, 2012 11:33 AM

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Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

Greater Yellowstone Area

406-333-4425

From: Clarke, Patrick R. - APHIS

Sent: Thursday, February 02, 2012 4:25 PM

To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: GonaCon Shopping List

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P. Ryan Clarke

USDA, APHIS, VS,WR

Regional Epidemiologist-GYA
Belgrade, MT
406-388-5162

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To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon Shopping List
Date: Tuesday, February 07, 2012 8:52:07 AM

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And if anyone has anymore acronym ideas, lets have them. I am tired of actually typing whole words for anything ☺

Rebecca Frey
Wildlife Biologist/Disease Specialist
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406-333-4425

From: McCollum, Matthew P - APHIS
Sent: Friday, February 03, 2012 4:18 PM
To: Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

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Sent: Friday, February 03, 2012 2:38 PM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

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Sent: Friday, February 03, 2012 11:33 AM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

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406-333-4425

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Sent: Thursday, February 02, 2012 4:25 PM

To: Frey, Rebecca K - APHIS; Rhyon, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: GonaCon Shopping List

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USDA, APHIS, VS,WR

Regional Epidemiologist-GYA

Belgrade, MT

406-388-5162

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon Shopping List
Date: Friday, February 03, 2012 4:17:49 PM

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Subject: RE: GonaCon Shopping List
Date: Friday, February 03, 2012 2:37:34 PM

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Subject: RE: GonaCon Shopping List
Date: Friday, February 03, 2012 11:33:02 AM

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Subject: RE: GonaCon Shopping List
Date: Thursday, February 02, 2012 4:27:53 PM

I have already sent that to Rick. Who else is asking or needs to be in the loop?

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Subject: RE: GonaCon Shopping List
Date: Thursday, February 09, 2012 8:35:23 AM

Ok, sounds good. Do one of you super smart peeps who might know someone at Biomark want to call and see what we can figure out? We know we have VHF collars and VIT's as well as RFID tags in ears. Lets see what they can recommend while trying to use/make adjustments to what we have. Thanks to whoever wants to volunteer!! Particularly since I went on their website and could not comprehend how a proximity data logger might fit into a VIT..... ☺

Becky

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406-333-4425

From: Nol, Pauline - APHIS
Sent: Tuesday, February 07, 2012 9:09 AM
To: McCollum, Matthew P - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: GonaCon Shopping List

Hiya, yeah, I'll bet Biomark would have some ideas. They use multiple sensing pit tags in bats too, etc.

We do need to keep in mind that a vag transmitter might come out in a different place from the fetus and the placenta. So we will likely have to hand tag the calf or associated pieces and parts.

From: McCollum, Matthew P - APHIS
Sent: Tuesday, February 07, 2012 9:03 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Shopping List

Good questions... Being in the "big idea" business, I really don't know much more yet. My thought is that there is technology out there that might work- gopher burrow sensors designed to record animals passing by them might be used for instance. I'm not sure if the tags would work as they are in the animals or if we'd have to put some in on the tip of their nose. I'd think we could incorporate a pit tag into a vag transmitter.

Biomark is one company that handles RFID implants, they might be helpful in putting something together.

Who's afraid of the big bad wolf? Ha ha ha ha haaa...

Matt

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Sent: Thursday, February 02, 2012 4:25 PM

To: Frey, Rebecca K - APHIS; Rhyen, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

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From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 4:39:00 PM

This get more sordid all the time!

Glad I'm not first author!!!;)

From: Rhyan, Jack C - APHIS
Sent: Tuesday, April 08, 2014 4:38 PM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: RE: GonaCon study bison-Montana

Hand emulsion. Apples and kumquots!

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 3:42 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: FW: GonaCon study bison-Montana

The last batch of Gonacon was made in the microfluidizer.

Do we want to use the same prep as the first group or use the hand emulsion prep?

From: Orahood, Darcy S - APHIS
Sent: Tuesday, April 08, 2014 2:35 PM
To: Nol, Pauline - APHIS
Subject: RE: GonaCon study bison-Montana

Hi Pauline,

Yes, according to my manufacturing and distribution records, this was the standard EPA-registered formulation (deer/horses) and it was indeed processed through the microfluidizer.

I'm waiting on some reagents to come in so I won't manufacture any vaccine until next week or the following week. I'll keep an eye out for your "official order" e-mail whenever you've decided on final numbers, volume, etc. ☺

Thanks,

DSO

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 2:07 PM
To: Orahood, Darcy S - APHIS
Subject: FW: GonaCon study bison-Montana

Hey Darcy,

Do you remember if you put this prep from 2 years ago through the emulsion machine or if you did it by hand?

We are aiming for the week of May 7th but still need to powwow about the prep before we place our official order☺

Thanks!

Pauline

From: Orahood, Darcy S - APHIS
Sent: Friday, March 30, 2012 8:34 AM
To: Nol, Pauline - APHIS; Miller, Lowell A - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: GonaCon study bison-Montana

I'll manufacture within the next 2 weeks and get with one of you to transfer the material once it is loaded in syringes, by Friday April 13th. Do you need any sham doses?

Thanks,

Darcy Orahood
Biological Science Technician
USDA National Wildlife Research Center
4101 LaPorte Ave
Fort Collins, CO 80521
(970) 266-6061

From: Nol, Pauline - APHIS
Sent: Wednesday, March 28, 2012 1:48 PM
To: Miller, Lowell A - APHIS; Orahood, Darcy S - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: GonaCon study bison-Montana



Hi Darcy and Lowell,

Our target date of vaccinating the bison up in Montana is April 15! Time has been flying for sure!

We would like 20 doses of 3000ug/syringe (3 ml) GonaCon by that time. Will that work with your schedules?

Thanks and let us know of any foreseeable problems or questions.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center

4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Orahood, Darcy S - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Miller, Lowell A - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Friday, March 30, 2012 8:34:00 AM

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From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 4:38:25 PM

Hand emulsion. Apples and kumquots!

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 3:42 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: FW: GonaCon study bison-Montana

The last batch of Gonacon was made in the microfluidizer.

Do we want to use the same prep as the first group or use the hand emulsion prep?

From: Orahood, Darcy S - APHIS
Sent: Tuesday, April 08, 2014 2:35 PM
To: Nol, Pauline - APHIS
Subject: RE: GonaCon study bison-Montana

Hi Pauline,

Yes, according to my manufacturing and distribution records, this was the standard EPA-registered formulation (deer/horses) and it was indeed processed through the microfluidizer.

I'm waiting on some reagents to come in so I won't manufacture any vaccine until next week or the following week. I'll keep an eye out for your "official order" e-mail whenever you've decided on final numbers, volume, etc. 😊

Thanks,

DSO

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 2:07 PM
To: Orahood, Darcy S - APHIS
Subject: FW: GonaCon study bison-Montana

Hey Darcy,

Do you remember if you put this prep from 2 years ago through the emulsion machine or if you did it by hand?

We are aiming for the week of May 7th but still need to powwow about the prep before we place our official order😊

Thanks!

Pauline

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Thanks and let us know of any foreseeable problems or questions.

Pauline

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Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Orahood, Darcy S - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 4:06:00 PM

Thanks Darcy. Do we still have hand emulsion capability?

From: Orahood, Darcy S - APHIS
Sent: Tuesday, April 08, 2014 2:35 PM
To: Nol, Pauline - APHIS
Subject: RE: GonaCon study bison-Montana

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Subject: FW: GonaCon study bison-Montana

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From: [Orahood, Darcy S - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Tuesday, April 08, 2014 2:34:58 PM

Hi Pauline,

Yes, according to my manufacturing and distribution records, this was the standard EPA-registered formulation (deer/horses) and it was indeed processed through the microfluidizer.

I'm waiting on some reagents to come in so I won't manufacture any vaccine until next week or the following week. I'll keep an eye out for your "official order" e-mail whenever you've decided on final numbers, volume, etc. 😊

Thanks,

DSO

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 2:07 PM
To: Orahood, Darcy S - APHIS
Subject: FW: GonaCon study bison-Montana

Hey Darcy,

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Sent: Wednesday, March 28, 2012 1:48 PM
To: Miller, Lowell A - APHIS; Orahood, Darcy S - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: GonaCon study bison-Montana



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Cell: (b) (6)
Fax: 970-266-6157

From: [Orahood, Darcy S - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Monday, April 14, 2014 4:52:32 PM

Hey Pauline,

As I mentioned earlier, we are only able to partially accommodate your hand-mixed emulsion request.

We can provide the primary emulsion (prepared using a vortexer) to you guys and then you are free to hand mix using a needle and syringes. Jack and Doug apparently discussed contacting Ken Crane to see if he had any interest in coming to the Center one day to assist with this since he used to do it regularly (and could probably be coerced with the promise of lunch or something along those lines). We can also provide you guys with the syringes, syringe caps, labels, and a large gauge blunt-tipped needle for loading the individual doses of vaccine.

However, we will need to coordinate so that whoever will be hand mixing the vaccine is available and ready to mix as soon as I finish preparing the vaccine. If the vaccine has to be stored in the refrigerator prior to hand mixing, the task become even more difficult than it was before! So it's best to just mix it right away and ideally, load the individual doses at that time, and then store in the refrigerator until use. You certainly can load the doses later but the vaccine is stiffer straight out of the fridge and I wouldn't recommend warming it to room temp once it has already been chilled.

GonaCon hasn't actually been emulsified in this fashion in over 5 years and we've never had any other special requests for this preparation so it has become quite a non-standard formulation!

I am hoping to make the vaccine next week (week of April 21st) so that there is plenty of time before you guys are ready to use the vaccine. Will you please let me know once you and Jack have talked and finalize who will be hand mixing the vaccine? Then we can schedule a day next week when I'll manufacture the vaccine and hand it over to the lucky mixer!

Thanks,

Darcy Orahood
Biological Science Technician
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone (970) 266-6061

From: Nol, Pauline - APHIS
Sent: Tuesday, April 08, 2014 4:06 PM
To: Orahood, Darcy S - APHIS
Subject: RE: GonaCon study bison-Montana

Thanks Darcy. Do we still have hand emulsion capability?

From: Orahood, Darcy S - APHIS
Sent: Tuesday, April 08, 2014 2:35 PM
To: Nol, Pauline - APHIS
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Cc: Rhyen, Jack C - APHIS; McCollum, Matthew P - APHIS
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(970) 266-6061

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To: Miller, Lowell A - APHIS; Orahood, Darcy S - APHIS
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Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Orahood, Darcy S - APHIS](#); [Miller, Lowell A - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: GonaCon study bison-Montana
Date: Friday, March 30, 2012 4:12:00 PM

Thank you Darcy!

And we do not need sham doses.

Pauline

From: Orahood, Darcy S - APHIS
Sent: Friday, March 30, 2012 8:34 AM
To: Nol, Pauline - APHIS; Miller, Lowell A - APHIS
Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
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Fax: 970-266-6157

From: [Patrick R Clarke](#)
To: [Rebecca K Frey](#)
Cc: [Jack C Rhyan](#); [Jason E Lombard](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Matt McCollum](#); [Pauline.Nol@aphis.usda.gov](#); [Rick_Wallen@nps.gov](#)
Subject: Re: GonaCon Study Call #2
Date: Tuesday, May 03, 2011 4:59:00 PM

Works for me too
P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell

From: Rebecca K Frey/MT/APHIS/USDA
To: Matt McCollum/CO/APHIS/USDA@USDA
Cc: Jack C Rhyan/CO/APHIS/USDA@USDA, Jason E Lombard/CO/APHIS/USDA@USDA, Jenny_Powers@nps.gov, Margaret_Wild@nps.gov, Patrick R Clarke/MT/APHIS/USDA@USDA, Pauline.Nol@aphis.usda.gov, Rick_Wallen@nps.gov
Date: 05/03/2011 04:05 PM
Subject: Re: GonaCon Study Call #2

works for me

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

Matt McCollum/CO/APHIS/USDA

05/03/2011 03:52 PM

To Jason E Lombard/CO/APHIS/USDA@USDA, Jack C Rhyan/CO/APHIS/USDA@USDA, Jenny_Powers@nps.gov, Margaret_Wild@nps.gov, Patrick R Clarke/MT/APHIS/USDA@USDA, Pauline.Nol@aphis.usda.gov, Rebecca.K.Frey@aphis.usda.gov, Rick_Wallen@nps.gov

cc

Subject GonaCon Study Call #2

Would it work for everyone to move our conference call back one hour from 10:00 Tuesday May 10 to 11:00? A conflict has come up for Jack at the 10:00 time.

Thanks,
Matt

From: [Rebecca K Frey](#)
To: [Matt McCollum](#)
Cc: [Jack C Rhyan](#); [Jason E Lombard](#); [Jenny Powers@nps.gov](#); [Margaret Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline.Nol@aphis.usda.gov](#); [Rick Wallen@nps.gov](#)
Subject: Re: GonaCon Study Call #2
Date: Tuesday, May 03, 2011 4:05:00 PM

works for me

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Emigrant, Montana
(406) 333-4425

Matt McCollum/CO/APHIS/USDA

05/03/2011 03:52 PM

To: Jason E Lombard/CO/APHIS/USDA@USDA, Jack C Rhyan/CO/APHIS/USDA@USDA, Jenny_Powers@nps.gov, Margaret_Wild@nps.gov, Patrick R Clarke/MT/APHIS/USDA@USDA, Pauline.Nol@aphis.usda.gov, Rebecca.K.Frey@aphis.usda.gov, Rick_Wallen@nps.gov

cc

Subject: GonaCon Study Call #2

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Thanks,
Matt

From: [Matt McCollum](#)
To: Rick_Wallen@nps.gov
Cc: [Jack C Rhyan](#); [Jason E Lombard](#); Jenny_Powers@nps.gov; Margaret_Wild@nps.gov; [Patrick R Clarke](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: RE: GonaCon Study Call #2
Date: Thursday, May 05, 2011 8:51:00 AM

Ok, its set up for 11:00 on Tuesday, May 10th.

Dial in numbers: (b) (6)

Participant passcode: (b) (6)

Thanks everyone,
Matt

-----Original Message-----

From: Rick_Wallen@nps.gov [mailto:Rick_Wallen@nps.gov]
Sent: Wednesday, May 04, 2011 10:47 AM
To: McCollum, Matthew P (APHIS)
Cc: Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); Jenny_Powers@nps.gov; Margaret_Wild@nps.gov; Clarke, Ryan P. (APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS)
Subject: Re: GonaCon Study Call #2

Matt, I have the same conflict that Jack has raised. Another conference call at 0900. We are likely to be finished around 1000 but the extra hour would help in the scheduling process. Rw

Matt.McCollum@aphis.usda.gov

To
05/03/2011 03:52 PM Jason.E.Lombard@aphis.usda.gov,
Jack.C.Rhyan@aphis.usda.gov,
Jenny_Powers@nps.gov,
Margaret_Wild@nps.gov,
Patrick.R.Clarke@aphis.usda.gov,
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Rick_Wallen@nps.gov
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Subject
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From: Rick_Wallen@nps.gov
To: Matt.McCollum@aphis.usda.gov
Cc: Jack.C.Rhyan@aphis.usda.gov; Jason.E.Lombard@aphis.usda.gov; Jenny_Powers@nps.gov; Margaret_Wild@nps.gov; Patrick.R.Clarke@aphis.usda.gov; Pauline.Nol@aphis.usda.gov; Rebecca.K.Frey@aphis.usda.gov
Subject: Re: GonaCon Study Call #2
Date: Wednesday, May 04, 2011 11:06:00 AM

Matt, I have the same conflict that Jack has raised. Another conference call at 0900. We are likely to be finished around 1000 but the extra hour would help in the scheduling process. Rw

Matt.McCollum@aph
is.usda.gov

To
05/03/2011 03:52 PM Jason.E.Lombard@aphis.usda.gov,
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Jenny_Powers@nps.gov,
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Pauline.Nol@aphis.usda.gov,
Rebecca.K.Frey@aphis.usda.gov,
Rick_Wallen@nps.gov
cc

Subject
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From: [Matt McCollum](#)
To: [Jason E Lombard](#); [Jack C Rhyan](#); [Jenny Powers@nps.gov](#); [Margaret Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline.Nol@aphis.usda.gov](#); [Rebecca.K.Frey@aphis.usda.gov](#); [Rick Wallen@nps.gov](#)
Subject: Re: GonaCon Study conference call April 26 at 11:00 AM
Date: Friday, April 15, 2011 4:28:00 PM

The call in number is (b) (6) and passcode is (b) (6).

Thanks much

,

Matt

From: Rick.Wallen@nps.gov
To: Matt.McCollum@aphis.usda.gov
Cc: Jack.C.Rhyan@aphis.usda.gov; Jason.E.Lombard@aphis.usda.gov; Jenny.Powers@nps.gov; Margaret.Wild@nps.gov; Patrick.R.Clarke@aphis.usda.gov; Pauline.Nol@aphis.usda.gov; Rebecca.K.Frey@aphis.usda.gov
Subject: Re: GonaCon Study conference call April 26 at 11:00 AM
Date: Monday, April 18, 2011 9:10:00 AM

Matt, thanks for organizing this conversation... I will participate in the call. RW

Matt.McCollum@aph
is.usda.gov

To
04/15/2011 04:28 PM Jason.E.Lombard@aphis.usda.gov,
Jack.C.Rhyan@aphis.usda.gov,
Jenny.Powers@nps.gov,
Margaret.Wild@nps.gov,
Patrick.R.Clarke@aphis.usda.gov,
Pauline.Nol@aphis.usda.gov,
Rebecca.K.Frey@aphis.usda.gov,
Rick.Wallen@nps.gov
cc

Subject
Re: GonaCon Study conference call
April 26 at 11:00 AM

The call in number is (b) (6) and passcode is (b) (6).

Thanks much
,
Matt

From: [McCollum, Matthew P - APHIS](#)
To: [Orahood, Darcy S - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon study info needed
Date: Monday, December 01, 2014 10:21:04 AM

Hey Darcy,

I'm thinking we got the samples to you and we got results back, but I can't find them on my computer. Would you mind sending a report to Becky?

Thanks!

Matt

From: Frey, Rebecca K - APHIS
Sent: Monday, December 01, 2014 10:14 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: GonaCon study info needed

Hi,

I am doing our annual report for YNP on the GonaCon study. Did you ever send the blood in from last January for GonaCon titers? I have not seen that report.

Thanks

From: [Orahood, Darcy S - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: GonaCon study info needed
Date: Monday, December 01, 2014 10:28:06 AM
Attachments: [QA-1858 Montana Bison xGnRH Titers 3.3.2014.xlsx](#)

Good morning,

Anti-GnRH titers for the YNP bison GonaCon study (QA-1858) are summarized in the attached spreadsheet. Please let me know if you have any questions!

Best regards,

Darcy Orahood

Biologist

National Wildlife Research Center

4101 LaPorte Avenue

Fort Collins, CO 80521

Phone (970) 266-6061

From: McCollum, Matthew P - APHIS
Sent: Monday, December 01, 2014 10:21 AM
To: Orahood, Darcy S - APHIS
Cc: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon study info needed

Hey Darcy,

I'm thinking we got the samples to you and we got results back, but I can't find them on my computer. Would you mind sending a report to Becky?

Thanks!

Matt

From: Frey, Rebecca K - APHIS
Sent: Monday, December 01, 2014 10:14 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: GonaCon study info needed

Hi,

I am doing our annual report for YNP on the GonaCon study. Did you ever send the blood in from last January for GonaCon titers? I have not seen that report.

Thanks

From: [Nol, Pauline \(APHIS\)](#)
To: Jenny_Powers@nps.gov
Cc: [Rhyan, Jack C \(APHIS\)](#); [Lombard, Jason E \(APHIS\)](#); Margaret_Wild@nps.gov; [McCollum, Matthew P \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); Rick_Wallen@nps.gov
Subject: RE: GonaCon Study
Date: Thursday, May 19, 2011 1:44:00 PM

Good catch Jenny. I have the correct numbers in the methods but forgot to change them in the animal care section.
Thank you!
Pauline

-----Original Message-----

From: Jenny_Powers@nps.gov [mailto:Jenny_Powers@nps.gov]
Sent: Wednesday, May 18, 2011 2:58 PM
To: Nol, Pauline (APHIS)
Cc: Rhyan, Jack C (APHIS); Lombard, Jason E (APHIS); Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R. (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov
Subject: RE: GonaCon Study

Great, this makes sense. Thanks for the clarification. So the only issue I see now is that the numbers in the ACUC protocol aren't completely consistent with what you have laid out here. Let's say you have minimal sample size of 16 seropositives and 4 seronegative animals per pasture.
This means either a total of 80 females plus 1 bull per pasture = 84 animals or at the upper end 96 females and 4 bulls for 100 total. Is this correct? Maybe the animal section at the end of the IACUC is per year?

Jenny

"Nol, Pauline
(APHIS)"
<Pauline.Nol@aphis.usda.gov> To
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>
05/18/2011 02:28 cc
PM "Margaret_Wild@nps.gov"
<Margaret_Wild@nps.gov>, "McCollum,
Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
"Clarke, Patrick R. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"Frey, Rebecca K (APHIS)"
<Rebecca.k.frey@aphis.usda.gov>,
"Rick_Wallen@nps.gov"
<Rick_Wallen@nps.gov>, "Lombard,
Jason E (APHIS)"
<Jason.E.Lombard@aphis.usda.gov>,
"Rhyan, Jack C (APHIS)"
<Jack.C.Rhyan@aphis.usda.gov>
Subject
RE: GonaCon Study

Hey Jenny,

At this point we are proposing two replicates of two pastures consisting of 16-18 seropositives and ~4-6 seronegatives. In one pasture, the seropositives will be vaccinated with Gonacon, and those in the second pasture will not be vaccinated. This would involve accumulating 36 seropositive animals for each replicate, so we will have to spend some time collecting enough seropositives for the second replicate. I hope this makes sense.

In summary-

Pasture A: 16-18 seropositive cows vaccinated with Gonacon
4-6 seronegative cows

Pasture B: 16-18 seropositive cows not vaccinated
4-6 seronegative cows

In 2012-2014 we will collect another 32-36 seropositive cows and 10-12 seronegatives to repeat the above design.

I think it will be valuable to monitor and record when the seronegatives become exposed/infected in the context of proof of concept, but I doubt we have enough numbers to do any kind of real time to event analysis do we?

Also, I recall that the question of quantifying shedding came up earlier.

Anyone correct me if I'm wrong, but as I see it, we will be measuring shedding in a yes/no format in the form of culture positive/negative swabs, milk, placenta, fetus etc. But we will also be able to quantify numbers of cfu/gram of milk, placenta, or fetal organs.

Lastly, we came up with the 5-10% abortion rate in the vaccinates because nothing is always 100%. We will likely get 100% in this study, but if we don't we still want to have the power to determine a difference between the two groups.

I hope I addressed everything alright.

Pauline

-----Original Message-----

From: Jenny_Powers@nps.gov [mailto:Jenny_Powers@nps.gov]

Sent: Tuesday, May 17, 2011 8:59 AM

To: Lombard, Jason E (APHIS); Rhyan_Wilcox@fws.gov; Jack__C__ <Jack.C.Rhyan@aphis.usda.gov/@nps.gov (APHIS)

Cc: Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R.

(APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov

Subject: Re: GonaCon Study

Hi Jason and Jack,

Thanks for looking into other potential study designs. Looks like we have some good advice to work with. I like the idea of looking at shedding particularly given their exposure occurred when they were still free ranging. A few sentinels makes sense from a proof of concept standpoint as long as we still have a control pasture. Did we decide on 3 treated pastures and 1 control? I can't remember now. I agree that it doesn't seem to make sense to cycle new sentinels in now that we are concentrating on shedding. I don't quite understand the survival or time to event aspects of your second paragraph. What do other folks think? Do we need another call or do I just need to take an epi primer?

Thanks again,
Jenny

"Lombard, Jason E
(APHIS)"
<Jason.E.Lombard@aphis.usda.gov> To
"McCollum, Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
05/13/2011 09:10 "Rick_Wallen@nps.gov"
AM <Rick_Wallen@nps.gov>
cc
"Rhyon, Jack C (APHIS)"
<Jack.C.Rhyon@aphis.usda.gov>,
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>,
"Margaret_Wild@nps.gov"
<Margaret_Wild@nps.gov>, "Clarke,
Ryan P. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"Nol, Pauline (APHIS)"
<Pauline.Nol@aphis.usda.gov>,
"Frey, Rebecca K (APHIS)"
<Rebecca.k.frey@aphis.usda.gov>
Subject
GonaCon Study

Hello,

I had the opportunity to discuss the study with 2 statisticians from CEAH yesterday and wanted to share with you what we discussed.

If we use seroconversion of sentinels as the outcome, we have to use pasture as the experimental unit and with only 4 pastures which we had talked about during the call, we have no power to detect any differences in seroconversion. A better option would be to measure shedding at the individual animal level (primarily seropositives) and with multiple samples per animal per sampling date and multiple dates, we will have repeated measures on each animal. We can also sample seronegatives for shedding and maybe will be able to detect shedding prior to seroconversion which I understand hasn't been fully investigated in bison. I talked with Jack and Matt yesterday and they were thinking about having 16-18 seropositives and 4 seronegatives per pasture which would give us plenty of animals to get a handle on shedding. I am not sure how you quantify shedding in animals that have aborted, had a stillborn or a normal calf where you have potentially large amounts of bacteria compared with animals that don't calve but I'm sure we can figure something out.

We had discussed replacing the control sentinels after all had seroconverted but I'm not sure that is necessary unless

we want to evaluate shedding in more non-vaccinated animals, besides we are only talking about 4 bison at this point. It might be more interesting to see if shedding increases as the proportion of seropositives in a pen increases, although with only 4 sentinels per pasture, 80% will be seropositive from the start. We could also look at it from a survival analysis or time to event perspective. When all the animals in the control pastures have seroconverted, we probably don't need to keep following them.

Cheers!

Jason

Jason E. Lombard, DVM, MS

Dairy Specialist / Veterinary Epidemiologist National Animal Health Monitoring System (NAHMS)

USDA:APHIS:VS:CEAH

2150 Centre Avenue, Bldg. B-2E7

Fort Collins, CO 80526-8117

phone 970.494.7245

fax 970.494.7228

From: [Pauline Nol](#)
To: [Matt McCollum](#)
Subject: Re: GonaCon Study
Date: Tuesday, April 26, 2011 8:34:00 AM

Yup at 11.

☐ [Matt McCollum](#)---04/15/2011 12:11:07 PM---So it looks like the 26th at 11:00 will work for most folks. I'll request a conference call and get

From: Matt McCollum/CO/APHIS/USDA
To: Jason E Lombard/CO/APHIS/USDA@USDA, Jack C Rhyan/CO/APHIS/USDA@USDA, Jenny_Powers@nps.gov, Margaret_Wild@nps.gov, Patrick R Clarke/MT/APHIS/USDA@USDA, Pauline.Nol@aphis.usda.gov, Rebecca.K.Frey@aphis.usda.gov, Rick_Wallen@nps.gov
Date: 04/15/2011 12:11 PM
Subject: Re: GonaCon Study

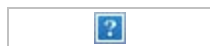
So it looks like the 26th at 11:00 will work for most folks. I'll request a conference call and get the information for everyone to access it. For now, here is the most recent version of the project document for your review.

[attachment "ImmunocontBisonProject_4-5-11.doc" deleted by Pauline Nol/CO/APHIS/USDA]

Thanks much,
Matt

☐ Re: GonaCon Study

Re: GonaCon Study



Jason E Lombard to: Matt McCollum

04/14/2011 04:37 PM

Cc: Jack C Rhyan, Jenny_Powers, Margaret_Wild, Patrick R Clarke, Pauline.Nol, Rebecca.K.Frey, Rick_Wallen

Hi Matt,

I'm available on the 26th at 11:00!

Thanks!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

 Matt McCollum/CO/APHIS/USDA

**Matt
McCollum/CO/APHIS/USDA**

04/14/2011 02:29 PM

ToMargaret_Wild@nps.gov,
Jenny_Powers@nps.gov,
Rick_Wallen@nps.gov, Jack C
Rhyan/CO/APHIS/USDA@USDA,
Pauline.Nol@aphis.usda.gov, Patrick R
Clarke/MT/APHIS/USDA@USDA,
Rebecca.K.Frey@aphis.usda.gov, Jason E
Lombard/CO/APHIS/USDA@USDA

cc


SubjectGonaCon Study

Hi folks,

We are wondering if there is a time in the not too distant future that we could all get together on the phone and talk about the gonacon study. I can set up a conference call if we can define a time that'd work. How about the week of April 25th? Can we put together a call for Tuesday April 26 at 11:00? If that time does not work for you, is there another time later in the week that would? If there is anyone else that you think I should have included in this, please let me know.

Thank you,

Matt McCollum

Wildlife Biologist
USDA-APHIS-VS-WRO
National Wildlife Research Center
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 - Office
 - Mobile
(970)266-6138 - Fax

"Whatever you are, be a good one." -Abraham Lincoln

From: [Matt McCollum](#)
To: [Jason E Lombard](#); [Jack C Rhyan](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline.Nol@aphis.usda.gov](#); [Rebecca.K.Frey@aphis.usda.gov](#); [Rick_Wallen@nps.gov](#)
Subject: Re: GonaCon Study
Date: Friday, April 15, 2011 12:11:00 PM
Attachments: [ATTMIS50.doc](#)

So it looks like the 26th at 11:00 will work for most folks. I'll request a conference call and get the information for everyone to access it. For now, here is the most recent version of the project document for your review.

(See attached file: ImmunocontBisonProject_4-5-11.doc)

Thanks much,
Matt

☐ Re: GonaCon Study

Re: GonaCon Study



Jason E Lombard to: Matt McCollum

04/14/2011 04:37 PM

Cc: Jack C Rhyan, Jenny_Powers, Margaret_Wild, Patrick R Clarke, Pauline.Nol, Rebecca.K.Frey, Rick_Wallen

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Jason

Jason E. Lombard, DVM, MS
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Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

☐ Matt McCollum/CO/APHIS/USDA

Matt

ToMargaret_Wild@nps.gov,

McCollum/CO/APHIS/USDA

04/14/2011 02:29 PM

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Clarke/MT/APHIS/USDA@USDA,
Rebecca.K.Frey@aphis.usda.gov, Jason E
Lombard/CO/APHIS/USDA@USDA

cc

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Matt McCollum

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Fort Collins, CO 80521
(970)266-6233 - Office
(b) (6) - Mobile
(970)266-6138 - Fax

"Whatever you are, be a good one." -Abraham Lincoln

Proposed Project:

DRAFT

Title: Evaluation of sterilization by use of and GonaCon™, an immunocontraceptive vaccine, and ovariectomy as means of decreasing the potential for transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

USDOI, NPS: Rick Wallen, Jenny Powers

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary to occasionally long term or permanent infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg GnRH compound. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing shedding and transmission of *B. abortus* which leads to persistence of the disease in populations.

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.
2. Evaluate the effect of sterilization produced by ovariectomy of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.

3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous or sterilization by ovariectomy has on *B. abortus* colonization in naturally-infected female bison; determine if pregnancies following infertility would result in non-infectious parturition.
4. Determine the effect of immune system stimulation via vaccination with Adjuvac (Gonacon's adjuvant) on brucella titers and shedding

Research Plan:

A total of at least 80 and not more than 100 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 40 to 60 seronegatives and 40 seropositives) and 8 seronegative bulls captured in late winter/spring 2011 and, if needed 2012 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility up to one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 80 female bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into four pastures, each containing 10 seropositives and 10 to 15 seronegative “sentinels” and 2 bulls. Seropositive bison in the four pastures will be treated as follows:

Pasture A (GonaCon treatment) will contain 10 seropositive females vaccinated with GonaCon™ vaccine (containing 3000µg), 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

Pasture B (Untreated control) will contain 10 seropositive female non-vaccinates, 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

Pasture C (Ovariectomized “gold standard control”) will contain 10 seropositive ovariectomized female bison, 10 to 15 seronegative female bison and 2 seronegative bulls.

Pasture D (Adjuvant-only treated controls) will contain 10 seropositive female bison treated with Adjuvac [™], 10 to 15 seronegative female bison and 2 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013 – 2017). Bulls will be separated from the cows after breeding season, from December until July. During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture. All bison will be tested by serology and culture in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate.

Spring 2012 – Apply treatments: 1. GnRH, 2. Adjuvac, 3. ovariectomize . Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2017 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

Conclusion of study – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. Determine the effectiveness of the permanent sterility produced by ovariectomy and temporary sterility produced by use of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds
2. Determine the effect of prolonged anestrus produced by GonaCon™ and ovariectomy on the survival of *B. abortus* in infected bison. Determine if contracepted female bison become shedders of *B. abortus* after resumption of reproduction.
3. Determine the effect of adjuvant alone on shedding and transmission in seropositive dams
4. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Appendix: Sample size calculation:

Pasture A Seroconversion	Sample size per group				
0.5	407	103	45	24	14
0.4	107	49	28	17	11
0.3	49	29	19	13	9
0.2	28	19	13	10	8
0.1	17	13	10	8	6
0.01	11	9	8	6	5
	0.6	0.7	0.8	0.9	0.99
	Pasture B Seroconversion				

It is anticipated in a fenced enclosure that a single abortion or shedding event will result in the infection of a majority of the sentinels, and no abortion or shedding event will result in no infection of the sentinels. Therefore a sentinel sample size of 10 to 15 should be adequate. Based on previous studies, at least 30% of young seropositive females are expected to abort in the course of 5 years.

From: [Rhyan, Jack C \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#)
Subject: RE: GonaCon Study
Date: Wednesday, May 18, 2011 3:46:37 PM

Good job! Thanks.
J

-----Original Message-----

From: Nol, Pauline (APHIS)
Sent: Wednesday, May 18, 2011 2:29 PM
To: Jenny_Powers@nps.gov
Cc: Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R. (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov; Lombard, Jason E (APHIS); Rhyan, Jack C (APHIS)
Subject: RE: GonaCon Study

Hey Jenny,

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In summary-

Pasture A: 16-18 seropositive cows vaccinated with Gonacon
4-6 seronegative cows

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Also, I recall that the question of quantifying shedding came up earlier. Anyone correct me if I'm wrong, but as I see it, we will be measuring shedding in a yes/no format in the form of culture positive/negative swabs, milk, placenta, fetus etc. But we will also be able to quantify numbers of cfu/gram of milk, placenta, or fetal organs.

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Sent: Tuesday, May 17, 2011 8:59 AM
To: Lombard, Jason E (APHIS); Rhyan_Wilcox@fws.gov; Jack_C__ <Jack.C.Rhyan@aphis.usda.gov/@nps.gov (APHIS)
Cc: Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov
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Cheers!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

From: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
To: [Nol, Pauline \(APHIS\)](mailto:Nol_Pauline@aphis.usda.gov)
Cc: [Rhyan, Jack C \(APHIS\)](mailto:Rhyan_Jack_C@aphis.usda.gov); [Lombard, Jason E \(APHIS\)](mailto:Lombard_Jason_E@aphis.usda.gov); Margaret_Wild@nps.gov; [McCollum, Matthew P \(APHIS\)](mailto:McCollum_Matthew_P@aphis.usda.gov); [Clarke, Patrick R. \(APHIS\)](mailto:Clarke_Patrick_R@aphis.usda.gov); [Frey, Rebecca K \(APHIS\)](mailto:Frey_Rebecca_K@aphis.usda.gov); Rick_Wallen@nps.gov
Subject: RE: GonaCon Study
Date: Wednesday, May 18, 2011 2:58:26 PM

Great, this makes sense. Thanks for the clarification. So the only issue I see now is that the numbers in the ACUC protocol aren't completely consistent with what you have laid out here. Let's say you have minimal sample size of 16 seropositives and 4 seronegative animals per pasture. This means either a total of 80 females plus 1 bull per pasture = 84 animals or at the upper end 96 females and 4 bulls for 100 total. Is this correct? Maybe the animal section at the end of the IACUC is per year?

Jenny

"Nol, Pauline
(APHIS)"
<Pauline.Nol@aphis.usda.gov> To
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>
05/18/2011 02:28 cc
PM "Margaret_Wild@nps.gov"
<Margaret_Wild@nps.gov>, "McCollum,
Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
"Clarke, Patrick R. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"Frey, Rebecca K (APHIS)"
<Rebecca_k_frey@aphis.usda.gov>,
"Rick_Wallen@nps.gov"
<Rick_Wallen@nps.gov>, "Lombard,
Jason E (APHIS)"
<Jason.E.Lombard@aphis.usda.gov>,
"Rhyan, Jack C (APHIS)"
<Jack.C.Rhyan@aphis.usda.gov>
Subject
RE: GonaCon Study

Hey Jenny,
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seropositives will be vaccinated with Gonacon, and those in the second pasture will not be vaccinated. This would involve accumulating 36 seropositive animals for each replicate, so we will have to spend some time collecting enough seropositives for the second replicate. I hope this makes sense.

In summary-

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4-6 seronegative cows

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In 2012-1014 we will collect another 32-36 seropositive cows and 10-12 seronegatives to repeat the above design.

I think it will be valuable to monitor and record when the seronegatives become exposed/infected in the context of proof of concept, but I doubt we have enough numbers to do any kind of real time to event analysis do we?

Also, I recall that the question of quantifying shedding came up earlier. Anyone correct me if I'm wrong, but as I see it, we will be measuring shedding in a yes/no format in the form of culture positive/negative swabs, milk, placenta, fetus etc. But we will also be able to quantify numbers of cfu/gram of milk, placenta, or fetal organs.

Lastly, we came up with the 5-10% abortion rate in the vaccinates because nothing is always 100%. We will likely get 100% in this study, but if we don't we still want to have the power to determine a difference between the two groups.

I hope I addressed everything alright.
Pauline

-----Original Message-----

From: Jenny_Powers@nps.gov [mailto:Jenny_Powers@nps.gov]

Sent: Tuesday, May 17, 2011 8:59 AM

To: Lombard, Jason E (APHIS); Rhyan_Wilcox@fws.gov; Jack__C__

<Jack.C.Rhyan@aphis.usda.gov/@nps.gov (APHIS)

Cc: Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov

Subject: Re: GonaCon Study

Hi Jason and Jack,

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Thanks again,
Jenny

"Lombard, Jason E
(APHIS)"
<Jason.E.Lombard@aphis.usda.gov> To
"McCollum, Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
05/13/2011 09:10 "Rick_Wallen@nps.gov"
AM <Rick_Wallen@nps.gov>
cc
"Rhyon, Jack C (APHIS)"
<Jack.C.Rhyon@aphis.usda.gov>,
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>,
"Margaret_Wild@nps.gov"
<Margaret_Wild@nps.gov>, "Clarke,
Ryan P. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"Nol, Pauline (APHIS)"
<Pauline.Nol@aphis.usda.gov>,
"Frey, Rebecca K (APHIS)"
<Rebecca.k.frey@aphis.usda.gov>
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Cheers!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist National Animal Health
Monitoring System (NAHMS) USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

From: [Jason E Lombard](#)
To: [Matt McCollum](#)
Cc: [Jack C Rhyan](#); [Jenny_Powers@nps.gov](#); [Margaret_Wild@nps.gov](#); [Patrick R Clarke](#); [Pauline.Nol@aphis.usda.gov](#); [Rebecca.K.Frey@aphis.usda.gov](#); [Rick_Wallen@nps.gov](#)
Subject: Re: GonaCon Study
Date: Thursday, April 14, 2011 4:37:00 PM

Hi Matt,

I'm available on the 26th at 11:00!

Thanks!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
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phone 970.494.7245
fax 970.494.7228

 Matt McCollum/CO/APHIS/USDA

**Matt
McCollum/CO/APHIS/USDA**

04/14/2011 02:29 PM

ToMargaret_Wild@nps.gov,
Jenny_Powers@nps.gov,
Rick_Wallen@nps.gov, Jack C
Rhyan/CO/APHIS/USDA@USDA,
Pauline.Nol@aphis.usda.gov, Patrick R
Clarke/MT/APHIS/USDA@USDA,
Rebecca.K.Frey@aphis.usda.gov, Jason E
Lombard/CO/APHIS/USDA@USDA

cc

SubjectGonaCon Study

Hi folks,

We are wondering if there is a time in the not too distant future that we could all get together on the phone and talk about the gonacon study. I can set up a conference call if we can define a time that'd work. How about the week of April 25th? Can we put together a call for Tuesday April 26 at 11:00? If that time does not work for you, is there another time later in the week that would? If there is anyone else that you think I should have included in this, please let me know.

Thank you,

Matt McCollum

Wildlife Biologist

USDA-APHIS-VS-WRO
National Wildlife Research Center
4101 Laporte Ave
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(970)266-6233 - Office
(b) (6) - Mobile
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From: [Nol, Pauline \(APHIS\)](#)
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Cc: Margaret_Wild@nps.gov; [McCollum, Matthew P \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); Rick_Wallen@nps.gov; [Lombard, Jason E \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#)
Subject: RE: GonaCon Study
Date: Wednesday, May 18, 2011 2:28:00 PM

Hey Jenny,

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Sent: Tuesday, May 17, 2011 8:59 AM
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Cc: Margaret_Wild@nps.gov; McCollum, Matthew P (APHIS); Clarke, Patrick R. (APHIS); Nol, Pauline (APHIS); Frey, Rebecca K (APHIS); Rick_Wallen@nps.gov
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"Margaret_Wild@nps.gov"
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USDA:APHIS:VS:CEAH

2150 Centre Avenue, Bldg. B-2E7

Fort Collins, CO 80526-8117

phone 970.494.7245

fax 970.494.7228

From: [Pauline Nol](#)
To: Jenny_Powers@nps.gov
Subject: Re: GonaCon Study
Date: Thursday, April 14, 2011 4:32:00 PM

And where are you off to now???

 [Jenny_Powers](mailto:Jenny_Powers@nps.gov)---04/14/2011 04:30:44 PM---Hi Matt,

Jenny_Powers@nps.gov

04/14/2011 04:30 PM

To: Matt.McCollum@aphis.usda.gov
cc: Jack.C.Rhyan@aphis.usda.gov,
Jason.E.Lombard@aphis.usda.gov,
Margaret_Wild@nps.gov,
Patrick.R.Clarke@aphis.usda.gov,
Pauline.Nol@aphis.usda.gov,
Rebecca.K.Frey@aphis.usda.gov,
Rick_Wallen@nps.gov

Subject: Re: GonaCon Study

Hi Matt,

Great idea. Unfortunately, I will be out of town April 25 - May 5. I can talk with Margaret and Rick before then and give them my ideas on the current version.

Thanks for setting this up,
Jenny

Jenny Powers, DVM
Wildlife Veterinarian
National Park Service
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525

Phone: [REDACTED]
Cell: (b) (6)
Fax: (970) 225-3585

Matt.McCollum@aphis.usda.gov

04/14/2011 02:29 PM

To: Margaret_Wild@nps.gov,
Jenny_Powers@nps.gov,
Rick_Wallen@nps.gov,
Jack.C.Rhyan@aphis.usda.gov,
Pauline.Nol@aphis.usda.gov,
Patrick.R.Clarke@aphis.usda.gov,
Rebecca.K.Frey@aphis.usda.gov,
Jason.E.Lombard@aphis.usda.gov

To

cc

Subject

GonaCon Study

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USDA-APHIS-VS-WRO
National Wildlife Research Center
4101 Laporte Ave
Fort Collins, CO 80521
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(b) (6) - Mobile
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To: Matt.McCollum@aphis.usda.gov
Cc: Jack.C.Rhyan@aphis.usda.gov; Jason.E.Lombard@aphis.usda.gov; Margaret_Wild@nps.gov;
Patrick.R.Clarke@aphis.usda.gov; Pauline.Nol@aphis.usda.gov; Rebecca.K.Frey@aphis.usda.gov;
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Date: Thursday, April 14, 2011 4:30:00 PM

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Jenny Powers, DVM
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1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525

Phone: (970) 267-2122
Cell: (b) (6)
Fax: (970) 225-3585

Matt.McCollum@aph
is.usda.gov

To
04/14/2011 02:29 PM Margaret_Wild@nps.gov,
Jenny_Powers@nps.gov,
Rick_Wallen@nps.gov,
Jack.C.Rhyan@aphis.usda.gov,
Pauline.Nol@aphis.usda.gov,
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Fort Collins, CO 80521
(970)266-6233 - Office
(b) (6) - Mobile
(970)266-6138 - Fax

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From: [Rebecca K Frey](#)
To: [Matt McCollum](#); [Margaret Wild](#); [Jenny Powers](#); [Rick Wallen](#); [Jack C Rhyan](#); [Pauline Nol](#); [Patrick R Clarke](#); [Jason E Lombard](#)
Subject: Re: GonaCon Study
Date: Thursday, April 14, 2011 3:36:00 PM

Works for me. Just let me know, I am flexible.
Becky Frey

 Matt McCollum---04/14/2011 02:29 PM MDT---

From: Matt McCollum
To: [Margaret_Wild@nps.gov](#); [Jenny_Powers@nps.gov](#); [Rick_Wallen@nps.gov](#); Jack Rhyan; Pauline Nol; Patrick Clarke; Rebecca Frey; Jason Lombard
Cc:
Date: 04/14/2011 02:29 PM MDT
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Rick_Wallen@nps.gov
Subject: Re: GonaCon Study
Date: Thursday, April 14, 2011 3:30:00 PM

Sounds like a good idea. That time works for me.

Margaret

Margaret A. Wild, DVM, PhD
Chief Wildlife Veterinarian
Biological Resource Management Division
National Park Service
1201 Oak Ridge Dr., Suite 200
Fort Collins, CO 80525
Office: (970) 225-3593
Cell: (b) (6)
Fax: (970) 225-3585

Matt.McCollum@aph
is.usda.gov

To
04/14/2011 02:29 PM Margaret_Wild@nps.gov,
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From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Stephens, Stephanie H - APHIS](#)
Subject: RE: GonaCon study
Date: Thursday, November 03, 2011 4:26:00 PM
Attachments: [Tribal letter GonaConStudyNoledits.docx](#)

Hi,
A few edits...take or leave.
Pauline

From: Rhyan, Jack C - APHIS
Sent: Thursday, November 03, 2011 11:49 AM
To: Stephens, Stephanie H - APHIS
Cc: Nol, Pauline - APHIS
Subject: GonaCon study

Stephanie,
Here it is. I'm ccing Pauline who will likely be able to improve the brief project writeup.
Jack



United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Washington, DC
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease shedding of *Brucella abortus*, the causative agent of brucellosis. We wanted to notify you of this potential project and changes to our animal disease regulations and are requesting your comments.

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APHIS plans to publish an environmental assessment concerning this project soon, final rule amending requirements for the interstate movement of livestock and poultry in title 9, Code of Federal Regulations (9 CFR). Specifically, we are considering changes to section 71.20, approval of livestock facilities, and section 71.21, tissue and blood testing at slaughter. The proposed changes, which are summarized below, will increase our ability to safeguard livestock and poultry through early detection and reduced spread of foreign, emerging, and domestic program diseases. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. Some of these animals were captured last spring and the remainder will be captured in upcoming years. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years. Half of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept separate. Animals will be maintained and abortions and births monitored. Seronegative bison will be placed with the seropositive contracepted animals and with the seropositive non-contracepted animals to evaluate transmission. Following each parturitionbirthing event, all bison will be examined for shedding of the *Brucella* organismbacteria.

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The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, *Brucella-negative* animals that meet the requirements for quarantine will be placed on tribal or public lands.

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Approval of Livestock Facilities (9 CFR 71.20)

Currently, to be an approved livestock market or to maintain approval, individuals legally responsible for the day-to-day operations of the livestock facility must meet certain conditions. Namely, they must sign an agreement entitled, "Approved Livestock Facility for Handling Livestock Pursuant to title 9 of the Code of Federal Regulations." In addition, they must keep records such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in, the



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facility. The records are required to be maintained for 2 years. The changes we are considering to the regulations would increase this recordkeeping requirement to 5 years.

Commented [DAR1]: if a proposed rule, say proposed

Tissue and Blood Testing at Slaughter (9 CFR 71.21)

Under 9 CFR 71.21, livestock or poultry moving interstate for slaughter or rendering can only be moved to a slaughtering or rendering establishment that has been approved and listed by the APHIS Administrator. For an establishment to be listed, the operator of the establishment must agree to a number of provisions, such as:

- Allowing APHIS and Food Safety and Inspection Service personnel, or APHIS contractors, access to the facility to take blood and tissue samples from animals at the facility
- Retaining individual identification of animals
- Providing office space with necessary furnishings, light, temperature control, and janitorial service

Under the current regulations, operators of slaughtering or rendering establishments are not required to sign an agreement, and there are no recordkeeping requirements for these establishments.

Commented [dcc2]: The current rule states that they must agree to allow us access, etc., in order to be listed by the Administrator so it is assumed they would agree in writing but the actual agreement is not in the current rule. I would change the wording to state that the agreement is not in the rule because they are already required to sign the agreement and many establishments have done so

Commented [DAR3]: True?

However, as amended in the draft final rule, operators of slaughtering and rendering establishments would be required to sign a listing agreement, if they move animals interstate. Owners or operators that do not move livestock or poultry interstate are not required to be listed. The agreement will show that operators agree to meet the requirements for listed slaughtering and rendering establishments. In the event of a disease outbreak, APHIS may need to collect samples at certain facilities. By having an agreement in place, it will be easier to detect and prevent the spread of foreign, emerging, or domestic animal diseases.

In addition, we are considering adding recordkeeping requirements. Owners and operators will be required to keep documents such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in the facility, for 5 years. Retaining these records, as well as those for approved livestock facilities, for 5 years will help us find potentially infected or exposed livestock or poultry more quickly and enable us to do a more in-depth traceback. Livestock owners will benefit from reductions in the time needed to find animals that have been exposed to disease, which may reduce the time needed for quarantines.

Commented [DAR4]: True?

Commented [dcc5]: Yes

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project's potential changes. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at Terry.W.Clark@aphis.usda.gov or by telephone at (919) 855-7167.

Commented [DAR6]: If we have proposed these changes, do we want to enclose the proposed rule for additional info?

Tribal Leader
Page 3

Sincerely,

John R. Clifford
Deputy Administrator

From: Jenny_Powers@nps.gov
To: [Lombard_Jason_E \(APHIS\)](mailto:Lombard_Jason_E@aphis.usda.gov); Rhyan_Wilcox@fws.gov; Jack__C__ <Jack.C.Rhyan@aphis.usda.gov/@nps.gov (APHIS)>
Cc: Margaret_Wild@nps.gov; [McCollum_Matthew_P \(APHIS\)](mailto:McCollum_Matthew_P@aphis.usda.gov); [Clarke_Patrick_R. \(APHIS\)](mailto:Clarke_Patrick_R@aphis.usda.gov); [Nol_Pauline \(APHIS\)](mailto:Nol_Pauline@aphis.usda.gov); [Frey_Rebecca_K \(APHIS\)](mailto:Frey_Rebecca_K@aphis.usda.gov); Rick_Wallen@nps.gov
Subject: Re: GonaCon Study
Date: Tuesday, May 17, 2011 8:59:36 AM

Hi Jason and Jack,

Thanks for looking into other potential study designs. Looks like we have some good advice to work with. I like the idea of looking at shedding particularly given their exposure occurred when they were still free ranging. A few sentinels makes sense from a proof of concept standpoint as long as we still have a control pasture. Did we decide on 3 treated pastures and 1 control? I can't remember now. I agree that it doesn't seem to make sense to cycle new sentinels in now that we are concentrating on shedding. I don't quite understand the survival or time to event aspects of your second paragraph. What do other folks think? Do we need another call or do I just need to take an epi primer?

Thanks again,
Jenny

"Lombard, Jason E (APHIS)"
<Jason.E.Lombard@aphis.usda.gov> To
"McCollum, Matthew P (APHIS)"
<Matt.McCollum@aphis.usda.gov>,
05/13/2011 09:10 "Rick_Wallen@nps.gov"
AM <Rick_Wallen@nps.gov>
cc
"Rhyan, Jack C (APHIS)"
<Jack.C.Rhyan@aphis.usda.gov>,
"Jenny_Powers@nps.gov"
<Jenny_Powers@nps.gov>,
"Margaret_Wild@nps.gov"
<Margaret_Wild@nps.gov>, "Clarke,
Ryan P. (APHIS)"
<Patrick.R.Clarke@aphis.usda.gov>,
"Nol, Pauline (APHIS)"
<Pauline.Nol@aphis.usda.gov>,
"Frey, Rebecca K (APHIS)"
<Rebecca_k_frey@aphis.usda.gov>
Subject
GonaCon Study

Hello,

I had the opportunity to discuss the study with 2 statisticians from CEAH yesterday and wanted to share with you what we discussed.

If we use seroconversion of sentinels as the outcome, we have to use pasture as the experimental unit and with only 4 pastures which we had talked about during the call, we have no power to detect any differences in seroconversion. A better option would be to measure shedding at the individual animal level (primarily seropositives) and with multiple samples per animal per sampling date and multiple dates, we will have repeated measures on each animal. We can also sample seronegatives for shedding and maybe will be able to detect shedding prior to seroconversion which I understand hasn't been fully investigated in bison. I talked with Jack and Matt yesterday and they were thinking about having 16-18 seropositives and 4 seronegatives per pasture which would give us plenty of animals to get a handle on shedding. I am not sure how you quantify shedding in animals that have aborted, had a stillborn or a normal calf where you have potentially large amounts of bacteria compared with animals that don't calve but I'm sure we can figure something out.

We had discussed replacing the control sentinels after all had seroconverted but I'm not sure that is necessary unless we want to evaluate shedding in more non-vaccinated animals, besides we are only talking about 4 bison at this point. It might be more interesting to see if shedding increases as the proportion of seropositives in a pen increases, although with only 4 sentinels per pasture, 80% will be seropositive from the start. We could also look at it from a survival analysis or time to event perspective. When all the animals in the control pastures have seroconverted, we probably don't need to keep following them.

Cheers!

Jason

Jason E. Lombard, DVM, MS
Dairy Specialist / Veterinary Epidemiologist
National Animal Health Monitoring System (NAHMS)
USDA:APHIS:VS:CEAH
2150 Centre Avenue, Bldg. B-2E7
Fort Collins, CO 80526-8117
phone 970.494.7245
fax 970.494.7228

From: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
To: [Nol. Pauline \(APHIS\)](#)
Subject: RE: GonaCon Study
Date: Thursday, May 19, 2011 2:18:53 PM

I am painfully sitting through an information committee meeting with Josh Dien at the moment. Want to come over and play cards??

Are you free tomorrow night? I decided to go to Sandra's for tamales. What the heck be spontaneous right?

J

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Stephens, Stephanie H - APHIS](#)
Subject: RE: GonaCon Tribal Letter
Date: Monday, November 07, 2011 10:43:00 AM
Attachments: [Tribal letter GonaCon 11.7.11Nol.docx](#)

Hi,

I just have typo corrections basically. I accepted all the previous track changes so all that's in this attached version is what I corrected.

Thanks!

Pauline

From: Rhyan, Jack C - APHIS
Sent: Monday, November 07, 2011 10:06 AM
To: Stephens, Stephanie H - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Tribal Letter

[Stephanie,](#)

[This looks good to me. Pauline may have some comments.](#)

[Thanks,](#)

[Jack](#)

From: Stephens, Stephanie H - APHIS
Sent: Monday, November 07, 2011 8:50 AM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: GonaCon Tribal Letter

Importance: High

Hi Jack and Pauline-

I've done some revisions to the draft tribal letter for the GonaCon study in Montana. There were a some comments internally from ERAS that the letter had some terms in it that might be better explained in simpler language. So the revisions I made have attempted to address this issue.

Can you please review the attached draft letter and let me know if it looks ok to you? If I've garbled anything important, please also correct me on that! Once you've commented, I'll get this letter back to Terry Clark so he can finalize and send it out.

Thanks,

Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134



United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Washington, DC
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease exposure to *Brucella abortus*, the bacteria that can cause -brucellosis. APHIS plans to publish an environmental assessment concerning this project soon. We wanted to notify you of this potential project and are requesting your comments.

One significant way that brucellosis can be spread between infected and uninfected bison happens when infected animals give birth. The materials associated with giving birth contain *Brucella abortus*, and uninfected bison often become exposed to the infected material. The study that APHIS wants to conduct will investigate one way to decrease the potential for this exposure to take place by preventing infected bison from giving birth.

Some of the animals that will be used in the study were captured last spring and the remainder will be captured in upcoming years. Blood samples will be collected from captured bison to test to see if there is evidence of brucellosis infection. Bison that test positive for the presence of brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows, and 8 seronegative bison bulls. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years.

In the proposed study, half of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the GonaCon® vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile from the vaccine, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept in separate areas during the study. Animals will be cared for throughout the study and abortions and births will be monitored. Seronegative bison will be placed with the seropositive GonaCon®-vaccinated animals and with the seropositive non-GonaCon®-vaccinated animals to evaluate transmission of brucellosis. Following each birthing event, all bison will be examined to see if they have produced infected materials that are capable of transmitting *Brucella abortus* to other bison.

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, animals that have tested negative for brucellosis that also meet the requirements for previously-established quarantine use will be placed on tribal or public lands.



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Tribal Leader
Page 2

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at Terry.W.Clark@aphis.usda.gov or by telephone at (919) 855-7167.

Sincerely,

John R. Clifford
Deputy Administrator

From: [Rhyan, Jack C - APHIS](#)
To: [Stephens, Stephanie H - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon Tribal Letter
Date: Monday, November 07, 2011 10:05:37 AM

Stephanie,
This looks good to me. Pauline may have some comments.
Thanks,
Jack

From: Stephens, Stephanie H - APHIS
Sent: Monday, November 07, 2011 8:50 AM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: GonaCon Tribal Letter
Importance: High
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Thanks,

Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: [Stephens, Stephanie H - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: GonaCon Tribal Letter
Date: Monday, November 07, 2011 11:25:48 AM

Pauline-Thanks so much for the quick review. I just sent the letter off to Terry Clark to get it signed and sent out, and I really appreciate your help in getting this done so efficiently!

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: Nol, Pauline - APHIS
Sent: Monday, November 07, 2011 10:44 AM
To: Rhyan, Jack C - APHIS; Stephens, Stephanie H - APHIS
Subject: RE: GonaCon Tribal Letter

Hi,

I just have typo corrections basically. I accepted all the previous track changes so all that's in this attached version is what I corrected.

Thanks!

Pauline

From: Rhyan, Jack C - APHIS
Sent: Monday, November 07, 2011 10:06 AM
To: Stephens, Stephanie H - APHIS; Nol, Pauline - APHIS
Subject: RE: GonaCon Tribal Letter

[Stephanie,](#)

[This looks good to me. Pauline may have some comments.](#)

[Thanks,](#)

[Jack](#)

From: Stephens, Stephanie H - APHIS
Sent: Monday, November 07, 2011 8:50 AM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: GonaCon Tribal Letter

Importance: High

Hi Jack and Pauline-

I've done some revisions to the draft tribal letter for the GonaCon study in Montana. There were a some comments internally from ERAS that the letter had some terms in it that might be better explained in simpler language. So the revisions I made have attempted to address this issue.

Can you please review the attached draft letter and let me know if it looks ok to you? If I've garbled anything important, please also correct me on that! Once you've commented, I'll get this letter back to Terry Clark so he can finalize and send it out.

Thanks,

Stephanie

Stephanie H. Stephens
USDA-APHIS-Environmental and Risk Analysis Services, Unit 149
Headquarters: 4700 River Road, Riverdale, MD 20737
Office Phone/Fax: (435) 658-5134

From: [McCollum, Matthew P - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: Green Ranch Quarantine Bison
Date: Wednesday, October 31, 2012 5:33:16 PM

I agree.
Sent from my handheld phone.

From: Frey, Rebecca K - APHIS
Sent: Wednesday, October 31, 2012 05:18 PM
To: Clarke, Patrick R. - APHIS; Herriott, Donald E - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: Green Ranch Quarantine Bison

So, I feel there is no possible way we can take those animals back. There will be brucellosis positive animals at every place, could be as early as Feb. if bison come out of the Park. Two of the locations have animals that WILL BE aborting, we can not have those animals in any kind of proximity in case birds etc. move fetal material around. They are silly to want to take them any closer to YNP in general.....they need to get away from here, not closer to the Hot Zone! I hope they don't try to push us from the top.....

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, October 31, 2012 1:44 PM
To: Herriott, Donald E - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: FW: Green Ranch Quarantine Bison

I spoke with Jorge this morning. Jorge was with the DOI group that we gave a tour of the GonaCon leases to on August 13, so he has an appreciation of the facilities we are using for the study. I discussed with him four things initially:

- a) We have or have had recently brucellosis positive animals at all three leases.
- b) We would have to think carefully about the placement of Green Ranch animals to make the risk of exposure as close to zero as possible
- c) The public perception will be that Green Ranch animal are being housed in with or next to positive animals
- d) We want to have room for the 2nd cohort of GonaCon animals when they come from the trap this winter.....2-3 months could mean 4-6 months especially with a new Governor.

Jorge said the alternative he is pushing is to keep them at the Green Ranch until the 2013 roundup, but they were exploring the other options.

Ryan

From: Silva-Banuelos, Jorge G [mailto:Jorge_Silva-banuelos@ios.doi.gov]
Sent: Wednesday, October 31, 2012 12:42 PM
To: Clarke, Patrick R. - APHIS
Subject: Green Ranch Quarantine Bison

Ryan –

It was a pleasure speaking with you today. As we discussed on the phone, the Green Ranch is planning to hold a roundup of the Yellowstone-origin quarantine bison in early December. The State of Montana would like to transfer 27 of those bison (calves of the year and possibly some yearlings) to the National Bison Range. During this roundup, samples will be collected to conduct genetic and health testing of the animals to determine which bison would be suitable for transfer to NBR and what level of NEPA compliance would be necessary. However, because the Green Ranch is only planning to hold one roundup this year, the 27 bison would also need to be loaded up onto a truck and moved off the Green Ranch or else they would need to stay there until the fall 2013 roundup. Because the FWS cannot move the quarantine bison to NBR until after the genetic results are back, this scenario would require relocating the 27 bison to another location for an interim period. While the State of Montana is checking with the Department of Livestock to determine the availability of a suitable location for this purpose, I also wanted to check with you to determine the availability and suitability of using a pasture at Corwin Springs (or another APHIS-managed facility) to hold these 27 bison for an interim period - likely somewhere between 2-3 months as the genetic results are expected to come back 6-8 weeks after the samples are sent to the lab. Obviously, a critical factor in determining the location's suitability is whether the bison can be placed there without compromising their brucellosis-negative status having gone through the QFS. But I defer to you and your colleagues to identify other factors that help to evaluate the site's suitability. I sincerely appreciate your willingness to look at this option further. If you have any other questions, please do not hesitate to contact me.

Thanks again,

Jorge Silva-Bañuelos | Special Assistant | Office of the Assistant Secretary for Fish & Wildlife and Parks

Department of the Interior | 1849 C Street NW | Room 3148 | Washington, DC 20240 | ☎ 202.208.6211 (direct)

jorge@ios.doi.gov

From: [redacted]
To: [redacted]
Subject: [redacted]
Date: [redacted]

Hey Beth,
Do you have the p agency data to the the wgl is Matt's ought back? We need to p eg check the appo entry as to one and i don't know who that is?
Thank!
P

Revised DVM MS 10
With a revised DVM MS 10, we
USDA, N-1000-10
National Wildlife Research Center
N-1000-10
1000-10
1000-10
1000-10
1000-10
1000-10

From: my Rebecca E-A HBS
Sent: Wednesday, May 19, 2010 11:28 AM
To: Matthew P-APIES
Cc: Beth A-HBS
Subject: [redacted]

Rebecca E-A
Wildlife Division
USDA
1000-10
1000-10
1000-10

From: Matthew P-APIES
Sent: Tuesday, May 18, 2010 11:28 AM
To: Rebecca E-A HBS
Cc: Beth A-HBS
Subject: [redacted]

Copy Attached for time

Form with handwritten entries and stamps. Includes sections for "ANIMALS TO BE MOVED", "ANIMALS TO BE RECEIVED", and "PART 1 - COMPANY SHIPMENT".

Sent from my iPhone

On Mar 18 2011 at 2:01 PM, Matthew P-APIES <[redacted]> wrote:

Here's the 1-27. Beth, we'll be moving once we load up and put a seal on.

Thanks & enjoyed
Matt

Sent from my iPhone

On Mar 18 2011 at 3:27 PM, Beth A-HBS <[redacted]> wrote:

Trish,

Let's manage this like an export permit. The number is 18002501, don't need to send a CPBW as soon as we see a livestock. Go ahead and send one in USA/here as usual.

Keith A. Roeder DVM
Colorado State Veterinary
305-250-1000

Please note that my email address has changed to [redacted]

On Tue Mar 18 2011 at 3:28 PM, Beth A-HBS <[redacted]> wrote:

Hi Beth,
Attached is the CVT from the house that Matt is bringing down from Montana. There will be 6 in total.
Matt will wait for the permit number and then email and image of the 1-27.
Thank!
Beth

Revised DVM MS 10
With a revised DVM MS 10, we
USDA, N-1000-10
National Wildlife Research Center
N-1000-10
1000-10
1000-10
1000-10
1000-10

—Original Message—
From: Matthew P-APIES
Sent: Tuesday, May 18, 2010 11:28 AM
To: Beth A-HBS
Subject: [redacted]

See attached.

Sent from my iPhone

This electronic message contains information generated by the USDA solely for the intended recipient. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the sender or recipient to criminal penalties. If you believe you have received this message in error, please notify the sender and delete the message immediately.

F to
To
Date
Subject

Super 1 Any backlogs a m using we can match up with ea tags on the shipment documents, no w
Thanks Kelly!

F to
To
Date
Subject

If these backlogs are all in the early lead better is 0790. The open cow is 0818 and the ewe cow is 0876.
Her radio tag is 155303.

If you are so no backlogs let me know I have a metal tag on.

F to
To
Date
Subject

F to
To
Date
Subject

Hey Kelly,
Do you have the p agency data for the th ewe I think I ought back? We need to p eg check the apps entry as to one and I don't know what it is.
Thank!

F to
To
Date
Subject

F to
To
Date
Subject

F to
To
Date
Subject

F to
To
Date
Subject

Copy Attached to time.

Form with handwritten entries and stamps. Includes fields for "ANIMALS TO BE MOVED", "ANIMALS TO BE QUARANTINED", and "ANIMALS TO BE SLAUGHTERED".

ANIMALS TO BE MOVED	ANIMALS TO BE QUARANTINED	ANIMALS TO BE SLAUGHTERED
1. DATE OF ARRIVAL 2. DATE OF DEPARTURE 3. DATE OF ARRIVAL 4. DATE OF DEPARTURE 5. DATE OF ARRIVAL 6. DATE OF DEPARTURE 7. DATE OF ARRIVAL 8. DATE OF DEPARTURE 9. DATE OF ARRIVAL 10. DATE OF DEPARTURE 11. DATE OF ARRIVAL 12. DATE OF DEPARTURE 13. DATE OF ARRIVAL 14. DATE OF DEPARTURE 15. DATE OF ARRIVAL 16. DATE OF DEPARTURE 17. DATE OF ARRIVAL 18. DATE OF DEPARTURE 19. DATE OF ARRIVAL 20. DATE OF DEPARTURE 21. DATE OF ARRIVAL 22. DATE OF DEPARTURE 23. DATE OF ARRIVAL 24. DATE OF DEPARTURE 25. DATE OF ARRIVAL 26. DATE OF DEPARTURE 27. DATE OF ARRIVAL 28. DATE OF DEPARTURE 29. DATE OF ARRIVAL 30. DATE OF DEPARTURE 31. DATE OF ARRIVAL 32. DATE OF DEPARTURE 33. DATE OF ARRIVAL 34. DATE OF DEPARTURE 35. DATE OF ARRIVAL 36. DATE OF DEPARTURE 37. DATE OF ARRIVAL 38. DATE OF DEPARTURE 39. DATE OF ARRIVAL 40. DATE OF DEPARTURE 41. DATE OF ARRIVAL 42. DATE OF DEPARTURE 43. DATE OF ARRIVAL 44. DATE OF DEPARTURE 45. DATE OF ARRIVAL 46. DATE OF DEPARTURE 47. DATE OF ARRIVAL 48. 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Send from my iPhone
On Mar 18 201 at 20 PM McColleen Mat low P- APRIS <mat.colleen@pennstate.edu> wrote

Here's the 1-27. Kelly will be in the morning once we load up and get a red on.

Thanks a bunch
Mat

Send from my iPhone

On Mar 18 201 at 3:27 PM Roder - CDA Ka th <ka.th@pennstate.edu> wrote

Trish,

Let's manage this like an export permit. The number is 180025-01, don't need to send a CPBW as soon as we see livestock. Go ahead and send one in USA/US as usual.

Keith A. Roder DVM
Colorado State University
950-226-156

Please note that my email address has changed to ka.th@pennstate.edu

On Tue Mar 18 201 at 3:26 PM McColleen - APRIS <mat.colleen@pennstate.edu> wrote
Hi Kai
Attached is the CTF from the h son that Mat is bringing down from Montana. There will be 6 h s to a lot.
Ma will be 1 for the permit number and then email and image of the 1-27.

Thank!
Pauline
Pauline Mc DVM MS PhD
Wildlife Health Investigation Team

USDA-APHIS-VS-STAS
National Wildlife Research Center
101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-246-6126
Cell: 970-246-6126
Fax: 970-246-6126

-----Original Message-----
From: McCullum, Matthew P., APHIS
Sent: Tuesday, March 18, 2014 3:13 PM
To: Neil Pauline, APHIS; Ryan, Jack C., APHIS
Subject: Health cert.

See attached.

Sent from my iPad

This electronic message contains information generated by the USDA solely for the intended recipients. Any unauthorized interception of this message or the use or disclosure of the information it contains may violate the law and subject the violator to civil or criminal penalties. If you believe you have received this message in error, please notify the sender and delete the message immediately.

From: [Patrick R Clarke](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: Immunocontraceptive project
Date: Tuesday, October 12, 2010 4:15:00 PM

9 am works for me...I think Becky is off tomorrow.

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell
□ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

10/12/2010 03:52 PM

ToPauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA

cc

SubjectImmunocontraceptive project

OK folks. Here tis. Please see what you think. Get out your red pencils. Pauline and I struggled a bunch with what to do with offspring. Do we keep them in with the adults for the entire study? Do we separate them prior to calving? The easiest thing would be to kill em before the next calving but what is the best science? I'd like to visit with yall tomorrow after you've had some ponderin' time. When's good? 9 am?

Jack

[attachment "ImmunocontBisonProject_10-12.doc" deleted by Patrick R
Clarke/MT/APHIS/USDA]

From: [Jack C Rhyan](#)
To: [Patrick R Clarke](#)
Cc: [Matt McCollum](#); [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: Immunocontraceptive project
Date: Tuesday, October 12, 2010 4:19:00 PM

That'll be handy, Becky being off while we plan her work. Call you at 9.
Jack

☐ Patrick R Clarke---10/12/2010 04:15:06 PM---9 am works for me...I think Becky is off tomorrow. P. Ryan Clarke, D.V.M.

**Patrick R
Clarke/MT/APHIS/USDA**

10/12/2010 04:15 PM

ToJack C Rhyan/CO/APHIS/USDA
ccMatt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA
SubjectRe: Immunocontraceptive project



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P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6)-cell

☐ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

10/12/2010 03:52 PM

ToPauline Nol/CO/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA,
Patrick R Clarke/MT/APHIS/USDA@USDA,
Rebecca K Frey/MT/APHIS/USDA@USDA
cc

SubjectImmunocontraceptive project

OK folks. Here tis. Please see what you think. Get out your red pencils. Pauline and I struggled a bunch with what to do with offspring. Do we keep them in with the adults for the entire study? Do we separate them prior to calving? The easiest thing would be to kill em before the next calving but what is the best science? I'd like to visit with yall tomorrow after you've had some ponderin' time. When's good? 9 am?

Jack

[attachment "ImmunocontBisonProject_10-12.doc" deleted by Patrick R Clarke/MT/APHIS/USDA]

From: [Fagerstone, Kathleen A \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Bens, Catherine M \(APHIS\)](#)
Subject: RE: Meeting about GonaCon Bison Project
Date: Thursday, June 30, 2011 8:19:39 AM

I am available today: after the seminar

Tomorrow: All Day

July 5: Before 1 or after 3

July 6: after 10:30 AM

July 7: all day except 11:30 – 1 PM

July 8: all day

From: Nol, Pauline (APHIS)
Sent: Wednesday, June 29, 2011 10:11 AM
To: Bens, Catherine M (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: Meeting about GonaCon Bison Project



Hi Cat and Kathy,

When would you both be available to talk about the best way to develop the NWRC protocol for our bison GonaCon study in Montana?

I'm available tomorrow and Friday and most of next week.

Thanks,

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126

Mobile: (b) (6)

From: [Nol, Pauline \(APHIS\)](#)
To: [Eisemann, John D \(APHIS\)](#); [Fagerstone, Kathleen A \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Miller, Lowell A \(APHIS\)](#); [O'Hare, Jeanette R \(APHIS\)](#)
Subject: RE: Meeting to discuss the Bison Study
Date: Friday, June 03, 2011 3:23:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 6.3.11.docx](#)

Here is the latest draft of QA1858. Please check on the regulatory requirements and corresponding appendices. I'll attach the approved ACUC once we are ready to submit. And I'll touch base with Cathy Bens before we do as well. Where I have comment balloons I was not sure what to fill in.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: Eisemann, John D (APHIS)
Sent: Friday, June 03, 2011 10:46 AM
To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)
Subject: Meeting to discuss the Bison Study

Jack and Kathy just set up a meeting at 2:00 pm (MT) to discuss the bison study. There are some important registration considerations that need to be discussed before the study planning goes too far. Hope you can make it. It will be in the conference room by my office. Stephanie, I will call you if you are available.

John D. Eisemann

National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 2 (Regulatory Considerations) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

*Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:_____ Date _____ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader _____ Date _____

Review and Processing:

QAU: _____ Date _____

Concur:

NWRC Assistant Director _____ Date _____

Approved:

NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ YELL-2011-SCI-5892 _____ May 10, 2011 _____ Permit(s) description _____ Number _____ Date _____
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
<input type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other: _____
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____
Test, Control and Reference Material/Devices		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

Commented [pn1]: ??

PART THREE: DESCRIPTION OF ACTIVITIES

Nature of the Collaboration: ☐ *Advisory Committee participation*
☒ *Manuscript/review article collaboration*
☐ *Training program requiring the use of animals*
☒ *Data analysis, interpretation and reporting*
☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date: _____

Commented [pn2]:

Anticipated Project Outcome: ☒ Manuscript
☒ Report
☐ Other: _____

Materials to be archived to close this activity: Raw data
Final Report

Description of Project and NWRC Activities and Participation: See research plan

Comments:

Attachments: IACUC Protocol Approval

(e.g. Material
Transfer Form,
IACUC approval,
etc.)

Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
 Proposed Experimental Termination Date: October 1, 2019
 Proposed Study Completion/Archive Date:

Commented [pn3]:

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

GonaCon Immunocontraceptive Vaccine for White-tailed Deer (*Odocoileus virginianus*): Pivotal target animal safety study Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland

Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer (*Dama dama*) at Point Reyes National Seashore, California

Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk (*Cervus elaphus*) at Rocky Mountain National Park, Colorado

Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota

Chemical sterilization of black-tailed deer

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

1. Immunocontraception of *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames, IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
 - a. Animal handling and sample collection records
 - b. Necropsy records
 - c. Results of serologic, histopathologic, and cultural analysis
 - d.
 - e.
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

Commented [pn4]:

	FY-xx	FY-xx	FY-xx	
A. Salary and Benefits				
B. Facilities (in addition to existing facility or space costs)				
C. Equipment				
D. Supplies				
E. Animal Care Costs				
F. Operating Costs (travel, misc. services, etc)				
TOTAL	\$0	\$0	\$0	

14. Human Health and Safety

HS004-00	Personal protective equipment
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15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. [J Wildl Dis.](#) 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. [Vet Rec.](#) 77:132-5.

[Robison, C. D.](#) D. S. [Davis](#), J. W. [Templeton](#), M. [Westhusin](#), W. B. [Foxworth](#), M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. [J Wildl Dis.](#) 34:582-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
 - ☒ Animal Use Appendix
 - ☐ Analytical Chemistry Appendix
 - ☐ Column E Explanation
 - ☐ Material Transfer Agreement
 - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
 - ☒ NEPA and ESA Appendix
 - ☒ Test, Control and Reference Material/Device Use Appendix
 - ☐ Other: (include appropriate title) _____

 - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

1) Animal Information: Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg

Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

14) Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

N. IACUC Approval

Date of IACUC Approval Letter: ACUC Protocol approved 5/17/2011_See attached

Commented [pn5]: By Montana IACUC-Name?

O. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects—internal or external—and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
 - ☐ B) not cause contaminants to enter water bodies
 - ☐ C) not adversely affect any federally protected species or critical habitat
 - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☒ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?
<input checked="" type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe species, potential impact and measures to be taken to minimize impact:
Consultations:
Did you consult with a state or federal agency specifically on this action.
<input type="checkbox"/> No
<input type="checkbox"/> Yes If yes, describe the date/mode/contact person and outcome of this consultation:
Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.
<input type="checkbox"/> No, permission not needed because:
<input type="checkbox"/> Yes

Commented [pn6]:

Commented [pn7]:

Test, Control and Reference Material/Devices Formulation and Use Appendix

A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
 - a) Concentration and purity: 1000ug/ml purity:na
 - b) Source: National Wildlife Research Center
 - c) Batch number: to be determined

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/KLH Conjugate (1000 µg)

Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
<i>Concholepas concholepas</i> hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml

AdjuVac™ adjuvant

<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: _____ Date: _____

D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

Commented [pn8]: ??

TRCS tracking number(s): _____

G. Material verification

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

Commented [pn9]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [O'Hare, Jeanette R \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#)
Subject: RE: Meeting to discuss the Bison Study
Date: Thursday, June 23, 2011 11:59:56 AM

Pauline,

I checked the GonaCon ingredients in the protocol. The only thing you might change is the water. It is really just "distilled water".

But I did not see anything related to "efficacy" per say. 1) I didn't see anything about GnRH titers. Is it in a later version or amendment? 2) Calving rates/pregnancy are necessary for your other study objectives, but not specifically mentioned in relation to GonaCon efficacy. If you have to write an amendment, maybe it could be related to the efficacy issue. Just a thought.

Let me know if you need anything.

Jeanette

From: Nol, Pauline (APHIS)
Sent: Friday, June 03, 2011 3:24 PM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study

Here is the latest draft of QA1858. Please check on the regulatory requirements and corresponding appendices. I'll attach the approved ACUC once we are ready to submit. And I'll touch base with Cathy Bens before we do as well. Where I have comment balloons I was not sure what to fill in.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: Eisemann, John D (APHIS)
Sent: Friday, June 03, 2011 10:46 AM
To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)
Subject: Meeting to discuss the Bison Study

Jack and Kathy just set up a meeting at 2:00 pm (MT) to discuss the bison study. There are some important registration considerations that need to be discussed before the study planning goes too far. Hope you can make it. It will be in the conference room by my office. Stephanie, I will call you if you are available.

John D. Eisemann

National Wildlife Research Center

4101 Laporte Avenue

Fort Collins, CO 80526

T: 970-266-6158

F: 970-266-6157

John.D.Eisemann@aphis.usda.gov

From: [Nol, Pauline - APHIS](#)
To: [Miller, Ryan S - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Farnsworth, Matthew L - APHIS](#); [Sweeney, Steven J - APHIS](#)
Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Date: Thursday, May 10, 2012 4:12:00 PM
Attachments: [PortfolioWILDIT4-3-08.doc](#)

Thanks Ryan!

I'm attaching what I think is the text from our portfolio that you wanted. Let me know if you need more.

Pauline

From: Miller, Ryan S - APHIS
Sent: Wednesday, May 09, 2012 4:19 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Cc: Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS
Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
All –

Great meeting with you guys today.

I am including a little additional information below about NIMBioS along with our working group proposal (attached) that was funded.

I'll contact Graham Hickling to arrange a time to chat about the idea.

Working Group: http://www.nimbios.org/workinggroups/WG_BovineTB

Workshop: http://www.nimbios.org/workshops/WS_BovineTB.html

More soon,

Ryan

From: Rhyan, Jack C - APHIS
Sent: Wednesday, May 09, 2012 10:13 AM
To: Miller, Ryan S - APHIS
Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
You bet!

J

From: Miller, Ryan S - APHIS
Sent: Wednesday, May 09, 2012 8:40 AM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS; Malmberg, Jennifer L - APHIS
Cc: Nol, Pauline - APHIS
Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
[Jack / Pauline does today at 10am work for you?](#)

Ryan

From: Rhyan, Jack C - APHIS
Sent: Tuesday, May 08, 2012 9:43 AM
To: Miller, Ryan S - APHIS; McCollum, Matthew P - APHIS; Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS; Malmberg, Jennifer L - APHIS
Cc: Nol, Pauline - APHIS
Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
I think so. Pauline is en route today so I'll wait for her to confirm, but lets tentatively plan it for tomorrow at 10 at NWRC.

Jack

From: Miller, Ryan S - APHIS
Sent: Tuesday, May 08, 2012 9:38 AM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Farnsworth, Matthew L - APHIS; Sweeney,

Steven J - APHIS; Malmberg, Jennifer L - APHIS

Cc: Nol, Pauline - APHIS

Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Great. Will 10am tomorrow (wed) work for you guys? We can come over to your place.

Ryan

From: Rhyan, Jack C - APHIS

Sent: Monday, May 07, 2012 3:20 PM

To: Miller, Ryan S - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS; Malmberg, Jennifer L - APHIS

Cc: Nol, Pauline - APHIS

Subject: FW: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
I think we are available Wed thru Friday this week.

Jack

From: Nol, Pauline - APHIS

Sent: Monday, May 07, 2012 3:17 PM

To: Rhyan, Jack C - APHIS

Subject: Re: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Hey Jack,

Yes, I'll be back in the office on Wednesday. Got samples today, all went well. Hope the conference went well last week.

P

From: Rhyan, Jack C - APHIS

Sent: Monday, May 07, 2012 02:02 PM

To: Nol, Pauline - APHIS

Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Hi!

Are you going to be back Thursday and Friday? We could suggest those days.

Jack

From: Nol, Pauline - APHIS

Sent: Thursday, May 03, 2012 2:35 PM

To: Miller, Ryan S - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS

Cc: Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS; Malmberg, Jennifer L - APHIS

Subject: RE: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Hi Ryan,

This sounds great! Jack, Matt, and I will all be on travel next week. I'll be around the week after next but tied up Tuesday, Wednesday, and Friday mornings.

Will wait to hear what works for everyone else.

Pauline

From: Miller, Ryan S - APHIS

Sent: Thursday, May 03, 2012 2:25 PM

To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS

Cc: Farnsworth, Matthew L - APHIS; Sweeney, Steven J - APHIS; Malmberg, Jennifer L - APHIS

Subject: Meeting to discuss the formation of a Livestock-Wildlife Interface team at CEAH.
Jack / Pauline –

Several of us (Matt, Steve, and I) have been tasked with developing a small team which will focused on addressing wildlife-livestock disease issues – primarily from an ecological / analytical perspective. This is a relatively unique opportunity here at CEAH, and something that we have been selling for several years. Our director is supportive of this role and wants us to further develop the function here at CEAH. As a result we are beginning to develop a strategic plan for this small group and scope projects for the next few fiscal years.

Since the two of you have been working in this area for years I'd like to meet to discuss how the

analytical work we focus on can best complement your work.

Would the two of you have time to meet in the next couple of weeks to discuss further?

Cheers,

Ryan

Ryan S. Miller

Ecologist

Centers for Epidemiology and Animal Health

USDA:APHIS:VS:CEAH

Phone: 970-494-7327

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Portfolio
Wildlife/Livestock Disease Investigations Team
2008

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Wildlife/Livestock Disease Investigations Team (WiLDIT)
(Located at the National Wildlife Research Center, Fort Collins, CO)

Administrative History

In 1997, following the '96/97 winter when over 1000 bison from Yellowstone National Park died or were sent to slaughter, Dr. Arnoldi, Deputy Administrator of APHIS/VS created the first position of the WiLDIT under the Western Regional Director, Dr. Bob Nervig. The position was located at the NWRC as part of the "One APHIS" concept. The purpose of the position was to help with GYA wildlife issues including continuing research projects begun in 1995. In 1999, when then Regional Director, Dr. Bill Buish, went to NVSL, Dr. Arnoldi placed the WiLDIT position under the VS Deputy's supervision and expanded the duties to include engagement in wildlife/domestic animal interface and gamefarm disease issues in which VS was involved. In 2000, Dr. Alfonso Torres replaced Dr. Arnoldi and reorganized his staff. He placed the position under Dr. Mike Gilsdorf of NCAHP. In 2001, Dr. Torres approved the WiLDIT to begin work on FMD in North American wildlife. In 2007 with Dr. Gilsdorf's retirement, NAHP was reorganized and the WiLDIT was placed under Dr. Diemer, the Assistant Regional Director of the Western Region and leader of the GYA core team.

Staff

Jack Rhyan DVM, MS - VMO

Matt McCollum - Wildlife Biologist

Pauline Nol DVM, MS - VMO; Attending Veterinarian for APHI/APHIS
wildlife facility

Karl Held - Animal Health Technician, APHI/APHIS wildlife facility

Leah Swanekamp - Animal Health Technician, Bison Quarantine Feasibility
Study in Montana, intermittent

Franklin Rigler - Animal Health Technician, Bison Quarantine Feasibility Study
in Montana, part-time

Sarah Coburn, MS - Veterinary student on Saul Wilson Scholarship;
scholarship provides part-time employment

Jon Pilon PhD - APHIS Science Fellow- shared with WS - administratively under
WS

Mission

The mission of the WiLDIT is to serve VS in the area of wildlife/livestock interface diseases:

- Serve with the GYA Core Team to promote and achieve the elimination of brucellosis from GYA bison and elk

- provide and disseminate knowledge of diseases at the wildlife/domestic animal interface to APHIS/VS and others
- liaise with state and federal agencies including wildlife and gamefarm authorities and NGOs.
- develop science-based solutions to disease problems at the wildlife/domestic animal interface.

Activity Areas

1. Consultation - Provide advice and consultation to Agency on interface disease issues; serve as liaisons with State and Federal wildlife agencies and NGO's. Serve as liaisons with WS.

2. Developmental work - Coordinate and/or conduct developmental work to address VS-specific problem areas, i.e. vaccine development for wildlife (brucellosis, TB, CWD, FMD), test diagnostics for wildlife (i.e., fluorescence polarization assay, infrared imaging technology), strategies to eliminate brucellosis from GYA wildlife (i.e., oral vaccination, immunocontraception, therapeutic vaccination, sustained release antibiotics). Developmental work is routinely done in collaboration with ARS, NWRC, and/or other federal agencies, CSU, and/or State wildlife agencies.

3. Monitoring/Surveillance - Conduct surveillance of wildlife around disease outbreaks or on continuing basis around endemic diseases, i.e. survey wildlife for TB around infected premises.

4. Training - Serve as training resource for the agency concerning interface diseases, i.e. trained contingent of VMOs on wildlife diseases in 2002. Serve as wildlife disease instructors at *Brucella* epi courses, FAD courses, CSU courses, and other Federal and State agency animal disease courses.

Funding

APHIS funding for WiLDIT has been primarily from the brucellosis account. Additionally, depending on the projects conducted, some funds have been transferred from TB funds, and from end-of-year money. Some projects have been funded, in part, by our collaborators from grants received from NSCREES, CSU, and NPS. Following are records of obligations for the past 5 years. These do not include salaries for Jack Rhyan and Matt McCollum.

	2004	2005	2006	2007	2008*
Salaries/benefits/overtime	26,000	52,000	67,500	147,000	201,000
Travel and transport	31,000	22,500	30,000	13,000	50,000
Rent/commun/utilities	50,000	50,000	80,000	74,000	50,000
Services	11,500	41,000	80,000	20,000	10,000
Supplies (scientific, feed, etc)	113,000	89,000	75,000	73,000	80,000
Equipment	27,500	3,000	8,500	3,000	0
Other	0	4,000	1,000	0	0
Total obligations	259,000	261,500	342,000	330,000	391,000

*Projected spending

Justifications

Why in Veterinary Services? In 1997, when the first position was established, Wildlife Services was not involved in any disease work with the exception of rabies. Since then, WS has developed a disease program including work on AI, TB, CWD, and continuing work in rabies. The VS Team has consistently liaised with WS in the development of the WS work and routinely collaborates with WS personnel in laboratory research and field work. This arrangement works well for both sister agencies. Examples of collaborative work include: CWD vaccine developmental work, immunocontraceptive development, sustained release antibiotic development, FLIR technology for animal disease surveillance, *Brucella* wildlife vaccine development, TB wildlife vaccine development, etc.

Why in Fort Collins? The Team's location at the NWRC is beneficial to both VS and WS. Additionally, the Fort Collins location allows frequent collaboration with other VS, ARS, CSU, DOI, and State domestic animal and wildlife disease experts. A valuable continuing relationship for the WiLDIT is that of the Animal Population Health Institute at CSU. The two entities share a wildlife disease research facility and routinely collaborate on projects. The CSU connection has been valuable in providing funding and students for needed disease studies. In addition, APHI has necessary, essential, specialized laboratory resources that WiLDIT personnel have utilized on collaborative projects.

Major Interface Disease Issues in which WiLDIT is Engaged

1. Brucellosis in GYA wildlife and feral swine: The primary disease issue the Team has been engaged in since its creation is the endemic infection of GYA bison and elk with *Brucella abortus*. The Team's involvement with this issue include: liaising with other involved agencies in several venues, collaborative research on the disease in wildlife and the risks posed to cattle, vaccine development for wildlife, serving as experts for agency epidemiologists in

development of risk assessments, cost-benefit analyses, decision memos, etc., and development of nonlethal strategies to eradicate the disease from GYA wildlife.

2. TB in Michigan deer: Team involvement in this issue includes wildlife surveillance around infected cattle premises, vaccine and vaccine marker development, bait development for vaccine delivery, and interagency planning of a future field trial in Michigan using the developed vaccine in deer.

3. CWD in cervids: Team involvement includes a 6 year study of CWD-exposed fallow deer and CWD vaccine development studies in mice and mule deer.

4. FMD in North American wildlife: WiLDIT personnel have collaborated with FADDL, ARS, and CSU to conduct FMD susceptibility and transmission studies in bison, elk, mule deer, and pronghorn. These studies were done at PIADC.

International Work

Poland: In 1998, WiLDIT personnel, at the request of the National Park Service visited Bialowieza National Park in Poland to consult with Polish animal health authorities on a disease condition in European bison in the Park.

KITA J., K. ANUSZ, M. ZALESKA, E. MALICKA, W. BIELECKI, B. OSINSKA, B. KOWALSKI, Z. KRASINSKI, A. DEMIASZKIEWICZ, J. RHYAN, M. KOLIPINSKI. 2003. Relationships among ecology, demography and diseases of European bison (*Bison bonasus*). Polish Journal of Veterinary Sciences 6: 261-266.

Israel: In 2007, WiLDIT personnel and APHIS/WS personnel were invited to Israel to observe FMD in Israeli wildlife and domestic animals and to field trial infrared imaging as a surveillance tool in a disease outbreak.

Workshop on Catastrophic Disease in Wildlife: In partnership with CSU/APHI, WiLDIT hosted "A Workshop on the Science of Surveillance, Control, and Eradication of Catastrophic Diseases in Wildlife." This workshop was held in August 2007 and involved 30 international wildlife disease experts. A report on the workshop was produced. A final product of the workshop which is in preparation will be a handbook designed for managers on the subject.

Students

WiLDIT personnel are adjunct staff members at CSU. In conjunction with CSU/APHI, WiLDIT assists in the education of numerous CSU students by providing project work for special-studies students, and graduate students. Additionally, WiLDIT offers externships for veterinary students and usually provides externships for 1 or 2 students annually. In the past, these have been from Washington, Ohio, Colorado, Virginia, and Brazil

**APHI/APHIS Wildlife Research Facility
(Hockaday/Swanson Wildlife Research Facility*)**

The **APHI /APHIS Wildlife Research Facility** occupies approximately 6.5 acres and is located on the Colorado State University Foothills Campus adjacent to the National Wildlife Research Center in Fort Collins, Colorado. The facility consists of multiple large paddocks with 8-foot high walls, while the entire perimeter of the site is surrounded by a 10-foot high fence. This facility contains handling equipment for deer, bison, elk, bighorn sheep, and other ungulate species. Security is provided by USDA/APHIS, National Wildlife Research Center and CSU security personnel. The animals and facility are maintained by a full time animal care staff and an attending veterinarian. All projects conducted at the facility are approved by the Colorado State University Animal Care and Use Committee.

*Constructed, equipped, and funded primarily with end-of-year money and excessed property.

List of Projects
(Project summaries in Appendix A)

- 1. Biosafety of RB51 in adult bison bulls.** Cooperative project with USDA/ARS/NADC.
- 2. Lesions and tissue colonization sites of *B. abortus* in aborted bison fetuses and adult female bison from YNP.** Cooperative study with ARS/NADC, MTFWP, MTDOL, and DOI.
- 3. Experimental infection of cattle with marine mammal isolate of *Brucella*** Cooperative study with Washington DNR and USDA/ARS/NADC.
- 4. Brucellosis epidemiology and pathogenesis:** Cooperative study with ARS/NADC, MTFWP, DOI/BRD, and DOI/NPS.
- 5. RB51 dose-response study in bison.** Cooperative project with USDA/ARS/NADC.
- 6. Safety of RB51 in nontarget species: Groundsquirrels, voles, ravens, deer mice, pronghorn, and black bears.** Cooperative projects with ARS/NADC, APHIS/WS, and CODOW.
- 7. RB51 persistence in the GYA.,** Cooperative project with ARS/NADC, MTFWP DOI, and CEAH.
- 8. Fetus disappearance in the GYA study** Cooperative project with MTFWP.

- 9. Contraception in bison (3 projects).** Cooperative studies with CSU and APHIS/WS.
- 10. Contraception in elk (2 projects).** Cooperative projects with WYG&F and NPS.
- 11. Sustained release rifampin as therapy for brucellosis in bison.** Cooperative projects with APHIS/WS and CSU/APHI.
- 12. *Brucella ovis* in Bighorn Sheep.** Cooperative study with CSU/APHI.
- 13. Risk of *Brucella* transmission to bison and cattle posed by bison or elk abortion events in nature.**
- 14. Bison quarantine feasibility study.** Cooperative project with Montana Fish, Wildlife and Parks, Montana Dept of Livestock and CSU/APHI.
- 15. Recombinant RB51 *Brucella* vaccine in elk 1.** Cooperative study with ARS/NADC and CSU/APHI.
- 16. Recombinant RB51 *Brucella* vaccine in elk 2 Oral.** Cooperative study with ARS/NADC and CSU/APHI.
- 17. Serologic differentiation of infection with *B. abortus* from *Yersinia enterocolitica* O:9 in elk.** Cooperative project with CSU/APHI, LSU, NVSL, NADC and CAFIA.
- 18. CWD susceptibility of fallow deer.** Cooperative study with ARS/NADC and Colorado DOW.
- 19. CWD vaccine studies (One mouse study and one deer study).** Cooperative studies with APHIS/WS, Colorado Division of Wildlife, and ARS.
- 20. Oral TB vaccines in white-tailed deer.** Cooperative project with CSU/APHI and ARS/NADC.
- 21. Development of low risk sheep/goats for weeds.** Cooperative project with CSU/APHI and ARS. Goats from this study are now used for weed control around quarantined bison at Fort Collins.
- 22. FMD in North American bison.** Cooperative study with FADDL and ARS.
- 23. FMD in elk.** Cooperative study with FADDL, ARS, and CSU/APHI.
- 24. FMD in pronghorn.** Cooperative study with FADDL, ARS, and CSU/APHI.

- 25. FMD in mule deer.** Cooperative study with FADDL, ARS, and CSU/APHI.
- 26. Stress response in captive-raised and wild-caught bighorn sheep.**
Cooperative project with CSU/APHI.
- 27. Infrared imaging for preclinical detection of FMD in mule deer.**
Cooperative study with FADDL, ARS, and CSU/APHI.
- 28. Infrared imaging for remote reading of TB skin tests in elk.** Cooperative project with APHIS/WS and CSU/APHI.
- 29. Evaluation of positive molecular vaccine markers expressed in *Mycobacterium bovis* BCG in a ruminant model: Pilot Project.** Cooperative study with CSU/APHI, USDA/ARS, Albert Einstein College of Medicine, and Michigan State University.
- 30. Evaluation of two interferon gamma assays for diagnosis of bovine tuberculosis (Cervigam and Bovigam) in captive cervids.** Cooperative study with CSU/APHI, USDA/ARS, and Prionics.

Future Work:

GYA Brucellosis

WiLDIT is committed to developing and implementing strategies to eradicate brucellosis from bison and elk in the GYA. Future work will determine on large samples sizes the duration of sterility produced by a single injection of GonaCon™, the immunocontraceptive vaccine. Future work will also produce a safe sustained-release antibiotic for decreasing *Brucella* infection in bison, and will explore the use of therapeutic vaccination in bison. These will conceivably be treatment modalities in a nonlethal strategy to eradicate brucellosis from bison. Developmental work in elk brucellosis will include further work in contraception, continued work on oral vaccination, and an "ecology of disease" study to produce a model for use in brucellosis elimination from elk. WiLDIT also will be involved in initiating and collaborating with CEAH and others on modeling efforts and cost-benefit analyses of brucellosis eradication.

TB in Cervids

WiLDIT has, in cooperation with CSU/APHI, obtained a grant to develop an immunologic marker for use in wildlife vaccines. Additionally, WiLDIT, in cooperation with APHIS/WS is developing baits for use in wildlife vaccines and is working toward a field trial in Michigan using the BCG formulation used in the previous white-tailed deer study. Current work is examining and comparing the use of Bovigam™ and Cervigam™, two tests for the analysis of gamma interferon, in elk.

CWD

In cooperation with APHIS/WS and CSU/APHI, WiLDIT has planned future work on the effect GnRH vaccine has on the development of CWD in mule deer. This is based on observations of GnRH use in elk that were exposed to CWD.

Feral Swine

WiLDIT, in cooperation with APHIS/WS and CSU/APHI, is developing an approach to dealing with diseases in feral swine. This approach utilizes temporary feeding sites, infrared surveillance for diseases, and pig specific feeders for poisoning, contracepting, or vaccinating feral swine. This approach might be utilized for several diseases including brucellosis, CSF, FMD, and influenza.

Wildlife Disease Surveillance

In cooperation with APHIS/WS, WiLDIT is involved in the development and validation of infrared surveillance for the screening of animals for certain diseases. These diseases include vesicular diseases in multiple species and febrile diseases in swine. The surveillance can be remotely conducted from fixed sites, and from manned and unmanned aircraft.

FMD

In cooperation with ARS, FADDL, and CSU/APHI, WiLDIT plans future studies to evaluate FMD vaccines for use in wildlife, specifically bison, white-tailed deer, and feral swine. The end-goal is the development of oral vaccines and delivery systems for use in wildlife for FMD and other diseases (CSF, ASF, etc.). These vaccines would be similar in application to the oral wildlife rabies vaccine currently in use.

From: [Clarke, Patrick R. - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [Thompson, Brent D - APHIS](#)
Subject: RE: Montana Brucellosis Projects
Date: Friday, March 21, 2014 8:47:01 AM

Pauline,

The problem with hosting students is that we have almost no notice as to when we're going to do any field work. When we immobilize or work trap bison or haze, etc. has very little lead time. I student could come to work with us in the "busy season" and just end up sitting around watching us work on the computer. I not telling you anything you don't already know.

The exception is when we run the GonaCon bison thru the chute...we have to set a date because of all the logistics involved.

Pauline, do have any sense of what (b) (6) actually wants to do? If she just wants to talk about the issues revolving around brucellosis in wildlife, we can certainly help out there,

Ryan

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Nol, Pauline - APHIS
Sent: Monday, March 10, 2014 10:38 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Thompson, Brent D - APHIS
Subject: FW: Montana Brucellosis Projects

Hey guys,

When things get back to "normal" for you, I wanted to see if you have any ideas regarding the student request below.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: (b) (6) [mailto:(b) (6)@gmail.com]

Sent: Saturday, March 08, 2014 1:56 PM

To: Dr. Pauline Nol; Nol, Pauline - APHIS

Subject: Montana Brucellosis Projects

Hello Dr. Nol,

I met you a few weeks ago at the WDA meeting. I am from a cow calf operation in Southwest Montana. Most of my experience is in cattle/sheep/horse management. This semester I am taking a Global Vet Public Health class and have become very interested in livestock disease control and surveillance. I am also keen on getting more involved with issues concerning livestock and wildlife health. I am interested in getting connected with veterinarians and other personel involved in Brucellosis screening in Montana and the GYE! Could you help me get in contact with someone?

Thank you!

(b) (6)

[REDACTED]
CSU DVM Candidate 2016
SCAVMA Hill's Pet Food Sales Associate
Agriculture Veterinary Education Team Coordinator
(b) (6) [@gmail.com](#)
406-209-2228

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: More stuff
Date: Monday, February 24, 2014 9:23:00 AM

Forgot to ask. Should I grab a hotel near the slaughter plant?

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 8:12 AM
To: Nol, Pauline - APHIS
Subject: RE: More stuff

Hi,

All the buckets are here. Hopefully the other parts arrive! The weather is really bad. However, I don't think there is any chance we will do 80 animals this week as Jack only stated to sample 2-5 YO's. So that may be 20-40 candidates, and we can only sample younger animals after we take our GonaCon animals. We might sample a few positive 2YO females that end up in GonaCon because we will be sampling before we know test status and we still need a lot of positives, but only 3 negatives. So, I might go ahead and sniff all of them, but we will take positives to GonaCon first.

Sound ok? Unless I can talk the park into stopping the negatives we don't need again.....

Rebecca Frey

Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Friday, February 21, 2014 1:03 PM
To: Frey, Rebecca K - APHIS
Subject: More stuff

Hey there,

So I think last trip I brought up around 70 "kits" for VOC collection (filters with tubes connected on either side). We theoretically need 80 but I ran out of filters at the time. I just put together some new filters thinking I would put more kits together and ship them up BUT I think I shipped the extra tubing and the connectors up already, silly me.

So....I'm going to ship more filters to you today. If it's possible to put a few more kits together before Wednesday, just in case you think you are able to collect 80 animals (Maybe that's possible with two people running the pumps and two bucket runners) then they will be available.

We can talk about this on Monday but I wanted to alert you to the fact that you'll be getting the filters, and another pump on Monday.

Did you get the buckets??

Thanks!

Pauline

[Pauline Nol, DVM, MS, PhD](#)
[Wildlife Livestock Disease Investigations Team](#)
[USDA-APHIS-VS-STAS](#)
[National Wildlife Research Center](#)
[4101 LaPorte Ave.](#)
[Fort Collins, CO 80521](#)
[Office: 970-266-6126](#)
[Cell: \(b\) \(6\)](#)
[Fax: 970-266-6157](#)

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: More stuff
Date: Monday, February 24, 2014 9:12:00 AM

Hey Becky,

Sounds good. Just get what is feasible, really appreciate you doing this!!

I forgot to tell you guys to have all the stuff with you when we do the video call. I realized I sent all my props out so all I've got is a bucket and some tubing, duh! It doesn't matter if the things I sent out Friday aren't there yet because they are just backup.

Looks like there will be a reprieve in the weather on Wednesday at least. So maybe I'll make it out. Was planning on being there Thursday and Friday to collect samples at slaughter. Is that still the case?

Talk soon. Hopefully this video thing will work! Otherwise we can skype or something on our own computers.

P

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 8:12 AM
To: Nol, Pauline - APHIS
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Thanks!

Pauline

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: More stuff
Date: Monday, February 24, 2014 8:11:51 AM

Hi,

All the buckets are here. Hopefully the other parts arrive! The weather is really bad. However, I don't think there is any chance we will do 80 animals this week as Jack only stated to sample 2-5 YO's. So that may be 20-40 candidates, and we can only sample younger animals after we take our GonaCon animals. We might sample a few positive 2YO females that end up in GonaCon because we will be sampling before we know test status and we still need a lot of positives, but only 3 negatives. So, I might go ahead and sniff all of them, but we will take positives to GonaCon first.

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Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

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To: Frey, Rebecca K - APHIS
Subject: More stuff

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Pauline

[Pauline Nol, DVM, MS, PhD](#)

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From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: More stuff
Date: Monday, February 24, 2014 1:02:25 PM

So, looks like we are testing tomorrow, shipping Wednesday, then testing more Wednesday to ship Thursday/ Friday. We are going to hold Brent up for slaughter sampling Wednesday if they don't all go to Ronan. Latest and greatest! Stay near the phone!

Becky
USDA APHIS VS
Sent from my iPhone

On Feb 24, 2014, at 9:23 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

[Forgot to ask. Should I grab a hotel near the slaughter plant?](#)

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 8:12 AM
To: Nol, Pauline - APHIS
Subject: RE: More stuff

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USDA APHIS Veterinary Services
Montana
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To: Frey, Rebecca K - APHIS
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Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: More stuff
Date: Monday, February 24, 2014 1:36:00 PM

Thanks for the update!

Call me tomorrow if any issues. I'll have two phones on me (b) (6) and (b) (6).

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 1:02 PM
To: Nol, Pauline - APHIS
Subject: Re: More stuff

So, looks like we are testing tomorrow, shipping Wednesday, then testing more Wednesday to ship Thursday/ Friday. We are going to hold Brent up for slaughter sampling Wednesday if they don't all go to Ronan. Latest and greatest! Stay near the phone!

Becky
USDA APHIS VS
Sent from my iPhone

On Feb 24, 2014, at 9:23 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

[Forgot to ask. Should I grab a hotel near the slaughter plant?](#)

From: Frey, Rebecca K - APHIS
Sent: Monday, February 24, 2014 8:12 AM
To: Nol, Pauline - APHIS
Subject: RE: More stuff

Hi,

All the buckets are here. Hopefully the other parts arrive! The weather is really bad. However, I don't think there is any chance we will do 80 animals this week as Jack only stated to sample 2-5 YO's. So that may be 20-40 candidates, and we can only sample younger animals after we take our GonaCon animals. We might sample a few positive 2YO females that end up in GonaCon because we will be sampling before we know test status and we still need a lot of positives, but only 3 negatives. So, I might go ahead and sniff all of them, but we will take positives to GonaCon first.

Sound ok? Unless I can talk the park into stopping the negatives we don't need again.....

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Friday, February 21, 2014 1:03 PM
To: Frey, Rebecca K - APHIS
Subject: More stuff

Hey there,

So I think last trip I brought up around 70 "kits" for VOC collection (filters with tubes connected on either side). We theoretically need 80 but I ran out of filters at the time. I

just put together some new filters thinking I would put more kits together and ship them up BUT I think I shipped the extra tubing and the connectors up already, silly me. So....I'm going to ship more filters to you today. If it's possible to put a few more kits together before Wednesday, just in case you think you are able to collect 80 animals (Maybe that's possible with two people running the pumps and two bucket runners) then they will be available.

We can talk about this on Monday but I wanted to alert you to the fact that you'll be getting the filters, and another pump on Monday.

Did you get the buckets??

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: [Mora, Darcy - APHIS](#)
To: [Wehtje, Morgan E - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: MT Bison serum
Date: Friday, October 28, 2016 4:42:11 PM

Morgan and Pauline,

I am finished with the MT bison neat sera and I returned all samples (3 boxes) to UC33 in lab B123 this afternoon.

Thanks again and have a wonderful weekend,

Darcy

From: Wehtje, Morgan E - APHIS
Sent: Thursday, October 27, 2016 4:00 PM
To: Mora, Darcy - APHIS <Darcy.Mora@aphis.usda.gov>
Cc: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: RE: MT Bison serum

Thanks, finding the samples was an excellent reminder that we need to organize our UCs a bit.

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/Vs/STAS/NVSL
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: Mora, Darcy - APHIS
Sent: Thursday, October 27, 2016 3:37 PM
To: Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>
Cc: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: RE: MT Bison serum

Morgan,

You rock – thanks very much to you and Pauline! I'll let you know if any questions come up as I begin aliquotting and diluting the sera.

Thanks,

Darcy

From: Wehtje, Morgan E - APHIS
Sent: Thursday, October 27, 2016 2:41 PM
To: Mora, Darcy - APHIS <Darcy.Mora@aphis.usda.gov>
Cc: Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: MT Bison serum

Darcy ,

The missing serum vials are in our UC in the VS freezer room (next door to the Gonacon room). They are on the top shelf of the freezer. The 2013 vials are large so maybe you just want to aliquot off what you need. There are not complete sets for each year. R08 is missing a 2013, Red15 , 30 and 23 are missing 2015 and Red 32 and higher do not have 2012 or 2013 draws. If you need anything else let me know.

Thanks

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
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4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
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Email: Morgan.E.Wehtje@aphis.usda.gov

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Morgan Wehtje, Wildlife Biologist
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Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: RE: my blurbs on our collaborative work with ARS
Date: Wednesday, February 01, 2012 9:12:00 AM

Since we told Stephanie Stevens that the Gonacon study did not involve ARS, could this become a problem somewhere?

From: Rhyan, Jack C - APHIS
Sent: Wednesday, February 01, 2012 9:08 AM
To: Nol, Pauline - APHIS
Subject: FW: my blurbs on our collaborative work with ARS

From: Herriott, Donald E - APHIS
Sent: Wednesday, February 01, 2012 9:06 AM
To: Rhyan, Jack C - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

From: Rhyan, Jack C - APHIS
Sent: Monday, January 23, 2012 1:29 PM
To: Herriott, Donald E - APHIS
Subject: RE: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

Don,

Please see [what you think about this](#).

Thanks,

Jack

From: Herriott, Donald E - APHIS
Sent: Monday, January 23, 2012 9:12 AM
To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Cc: Frey, Rebecca K - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
[Can you send me a "gonacon study summary page" asap?](#)
thx

From: Davidson, Mark L - APHIS
Sent: Monday, January 23, 2012 8:58 AM
To: Herriott, Donald E - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
[See below, too quick with send button](#)

From: Davidson, Mark L - APHIS
Sent: Monday, January 23, 2012 8:58 AM
To: Burke L Healey
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
[Burke, Can you check with Kevin and see if we need any new support from ARS on CFTEP or there are particular items we want to highlight for continued support?](#)
[Don, Anything GYA?](#)
[Will need the info, sometime this week.](#)

From: Dick, Jere L - APHIS
Sent: Friday, January 20, 2012 1:12 PM
To: Anelli, Joseph F - APHIS; McCluskey, Brian J - APHIS; Shere, Jack A - APHIS; Davidson, Mark L - APHIS
Cc: Fisher, Sharon S - APHIS; Christensen, Laura C - APHIS
Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12
[Do any of you have any ARS specific items that need to be discussed at the Senior Management level?](#)

From: Fisher, Sharon S - APHIS

Sent: Friday, January 20, 2012 3:10 PM

To: APHIS-VS Executive Team

Subject: FW: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

Hi all – We are need of items for APHIS-ARS meeting. Seems like there should be something what with all the NBAF and pre-harvest activity of late. Please let me know by TUESDAY next week.

From: Lewandowski, Laura P - APHIS

Sent: Friday, January 20, 2012 8:47 AM

To: Christensen, Laura C - APHIS; Cooper, Julie F - APHIS; Fisher, Sharon S - APHIS; Griffith, Carol A - APHIS; Hancock, Jason M - APHIS; Joyce, Carrie E - APHIS; Lohs, Christina L - APHIS; Mahoney, Bridget C - APHIS; Myers, Christina J - APHIS; Nesbitt, Thomas C - APHIS; Shelor, Steven L - APHIS; Tuck, James M - APHIS; White, Robin L - APHIS

Cc: Barsi, Janel L - APHIS

Subject: ACTION: Call for Agenda Items for APHIS/ARS Meeting, 2/3/12

The next bimonthly APHIS/ARS meeting is scheduled for Friday February 3, 2012 from 1:00 – 2:00 in conference room 305-E. In advance of that meeting, please send me any of your suggested agenda items by cob Thursday January 26. We will schedule a pre-meeting for sometime during the week of January 23 so I'll be in touch on that.

Thanks!

Laura Porcella Lewandowski

Acting Deputy Chief of Staff

Animal and Plant Health Inspection Service

1400 Independence Avenue, SW

Jamie L. Whitten Building, Room 314-E

Washington, D.C. 20250

202- 690-2288 (Monday, Wednesday, Friday)

301-436-3185 (Tuesday and Thursday)

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Naltrexone
Date: Monday, June 24, 2013 2:45:27 PM

(b) (6)

Thanks!

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Monday, June 24, 2013 2:45 PM
To: Frey, Rebecca K - APHIS
Subject: RE: Naltrexone

Hi Becky
Best shipping address?
P

From: Frey, Rebecca K - APHIS
Sent: Monday, June 24, 2013 2:44 PM
To: Nol, Pauline - APHIS
Subject: Naltrexone

Hi there!

We are out of Naltrexone.....need to dart 3 more cows....and maybe a few bulls before it is over. On the last bottle of A3080 now, have 8ml left so fingers crossed that lasts.

As soon as we can get Naltrex we will need it as 2 of these cows could calve anytime.
Thanks

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Brian J. McCluskey](#)
To: [Pauline Nol](#)
Subject: Re: Narrative for TBCAP deer vaccine project
Date: Wednesday, June 16, 2010 2:50:00 PM

I have posed the question on you receiving these funds to Bobbie Purcell. She is with Marketing and Regulatory Programs business services. She indicated she would look into it.

Brian J. McCluskey, DVM, MS, PhD, Dip. ACVPM
Director, Veterinary Services, Western Region
Fort Collins, CO
970.494.7385

☐ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

To: Brian J McCluskey/CO/APHIS/USDA@USDA
cc

Subject: Narrative for TBCAP deer vaccine project

06/16/2010 02:44 PM

This is the "safety of BCG in deer" proposal I'm submitting for TB CAP, We are also submitting another one jointly with Kurt VerCauteren here at NWRC dealing with vaccine delivery but that might as well go through him.

Thanks again.

Pauline

[attachment "TB CAP Wildlife Vaccine Narrative PNol.docx" deleted by Brian J McCluskey/CO/APHIS/USDA]

From: [Greiner, Laura B - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: New scan of Gonacon ACUC
Date: Tuesday, February 21, 2012 12:54:27 PM

Thanks, I think it is ready to forward to the Director's office!

Laura Greiner

Quality Assurance Specialist | 970-266-6022 | laura.b.greiner@aphis.usda.gov
National Wildlife Research Center | 4101 LaPorte Avenue | Fort Collins, CO 80521

From: Nol, Pauline - APHIS
Sent: Tuesday, February 21, 2012 12:53 PM
To: Greiner, Laura B - APHIS; Greiner, Steven J - APHIS
Subject: New scan of Gonacon ACUC



I ended up rescanning it anyway☺

Let me know if it doesn't look right again.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

[illegible]

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

Pauline
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
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Ryan
P Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

Becky
PS... I have jury duty tomorrow... cross your fingers it doesn't last through next week... or maybe that it does... either way, I will understand ;-)
Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

Pauline
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
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4101 LaPorte Ave.

Fort Collins, CO 80521
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From: [Pauline Nol](#)
To: [Jack C Rhyan](#)
Cc: [Matt McCollum](#); [Rebecca K Frey](#)
Subject: Re: "nuther draft of contraception protocol"
Date: Tuesday, February 01, 2011 5:11:00 PM
Attachments: [ImmunocontBisonProject_2-1-11NolEdits.doc](#)

I just made some small edits that are not related to content.

Is it acceptable at this point to keep the "conservation" term that squishy? Will someone railroad us on it potentially before we have it outlined? Although I guess having the process spelled out in great detail doesn't stop the PTB from doing whatever they want anyway. Oops, I think that was cynicism.

P

(See attached file: ImmunocontBisonProject_2-1-11NolEdits.doc)

☐ Jack C Rhyan---02/01/2011 02:57:50 PM---Please check it out and make any suggestions. this one has both seronegative calves and adults that pass quarantine being used

**Jack C
Rhyan/CO/APHIS/USDA**

ToRebecca K Frey/MT/APHIS/USDA@USDA,
Matt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA

02/01/2011 03:02 PM

cc

Subject'nuther draft of contraception protocol

Please check it out and make any suggestions. this one has both seronegative calves and adults that pass quarantine being used for conservation. The devil is in the details so we will have to carefully design the process by which the critters are "used for conservation."

Jack

[attachment "ImmunocontBisonProject_2-1-11.doc" deleted by Pauline Nol/CO/APHIS/USDA]

Proposed Project:

DRAFT

Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

Investigators:

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three

abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for B. abortus after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. {The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.} Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at -minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December til July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

From: [Matt McCollum](#)
To: [Jack C Rhyan](#)
Cc: [Pauline Nol](#); [Rebecca K Frey](#)
Subject: Re: "nuther draft of contraception protocol"
Date: Wednesday, February 02, 2011 9:28:00 AM
Attachments: [bison contraception study mm.doc](#)

Looks good. Just a couple suggestions.

Matt

(See attached file: bison contraception study mm.doc)

☐ Jack C Rhyan/CO/APHIS/USDA

**Jack C
Rhyan/CO/APHIS/USDA**

02/01/2011 03:02 PM

To: Rebecca K Frey/MT/APHIS/USDA@USDA,
Matt McCollum/CO/APHIS/USDA@USDA,
Pauline Nol/CO/APHIS/USDA@USDA

cc

Subject: nuther draft of contraception protocol

Please check it out and make any suggestions. this one has both seronegative calves and adults that pass quarantine being used for conservation. The devil is in the details so we will have to carefully design the process by which the critters are "used for conservation."

Jack

[attachment "ImmunocontBisonProject_2-1-11.doc" deleted by Matt McCollum/CO/APHIS/USDA]

Proposed Project:

DRAFT

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3. Evaluate *B. abortus* transmission to bison bulls during rut.

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3.4. Provide brucella negative animals to conservation herds.

Research Plan:

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the ~~facilities~~ facilities. These facilities comprise ~~7~~ 5 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

Pasture A will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Pasture B will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December until July. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements ~~as~~ published in the UM&R will be used for bison conservation. (The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.) Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at- minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

Time line:

Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

Spring 2012 – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December ~~til~~ through July each year.

Summer 2015 – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults ~~and~~ offspring meet requirements of quarantine, use for bison conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

5.6. Animals that pass the quarantine requirements will be incorporated into conservation herds.

Formatted: List Paragraph, No bullets or numbering

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, January 07, 2015 12:46:57 PM

Did we happen to pickle some of the placenta? I found calf tissues but no placenta.

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Wednesday, January 07, 2015 12:38 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

The placenta was not only "contaminated", it was disgusting!

-----Original Message-----

From: Rhyan, Jack C - APHIS
Sent: Wednesday, January 07, 2015 12:33 PM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS
Sent: Wednesday, January 07, 2015 12:26 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 14-029714

Date Received: 09/16/2014 09:30:41 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: MT

Species: [Bison]

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: RE: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov
Date: Wednesday, January 07, 2015 1:57:58 PM

Don't recall. I kinda doubt it.

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Wednesday, January 07, 2015 12:59 PM
To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

Hmmmm. I don't think we did. Matt, do you remember?

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team USDA-APHIS-VS-STAS National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

-----Original Message-----

From: Rhyan, Jack C - APHIS
Sent: Wednesday, January 07, 2015 12:47 PM
To: Nol, Pauline - APHIS; Nol, Pauline - APHIS
Subject: RE: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to Jack.C.Rhyan@aphis.usda.gov,Matt.McCollum@aphis.usda.gov,pauline.nol@aphis.usda.gov,Rebecca.K.Frey@aphis.usda.gov

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-----Original Message-----

From: APHIS-NVSL Case Coordinator - APHIS

Sent: Wednesday, January 07, 2015 12:26 PM

To: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS

Subject: NVSL Report - Accession#14-029714,Purpose:DEV_RES,Exam Req:BRUC sent to

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Submitter Name: Jack C Rhyan

Submitter Company: USDA, APHIS, VS

National Wildlife Research Center

Referral Number:

FAD Number:

Accession: 14-029714

Date Received: 09/16/2014 09:30:41 AM

Purpose: Developmental Research

Exam(s) Requested: BRUC

Submitter State: CO

Owner State: CO

Animal State: MT

Species: [Bison]

From: [Frey, Rebecca K - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: other permit question
Date: Wednesday, November 06, 2013 5:53:28 PM

Not sure. Think we are covered for Gonacon but I will check. Still trying to corner the corral crew on the sniffer, but got a tentative nod from Hallac when Ryan mentioned it.

Becky
USDA APHIS VS
Sent from my iPhone

On Nov 6, 2013, at 11:33 AM, "Rhyan, Jack C - APHIS" <Jack.C.Rhyan@aphis.usda.gov> wrote:

While we're on it, do we need to do anything for collecting bison next spring for the contraception study or is that already covered?

Jack

From: [Rebecca K Frey](#)
To: [Pauline Nol](#)
Subject: Re: plot gets thicker.....
Date: Wednesday, March 09, 2011 6:24:00 PM

Hey,

So.....as for numbers. We can prpbaly easily hold up to 40 per group....except for the "controls" and I should try to get away with as few of those as possible. The only limiting factor will be projected calves, because we can run out of space quickly, and if we need to keep animals longer to find out more data, that will mean more calves. The bull calves will be easily passed through quarantine, and we are planning on picking up the SlipnSlide facility once the actual quarantine animals leave. We would however need to also hang on to Brogans if we are talking about keeping more animals for longer. Maybe NPS could pick up that lease. I will check with Brian to see how many we can afford to feed!

I think we could use Kammy Johnson as our stats monster....she is working with us on the bull study and will be much easier to coerce than an outside party. And she is really good.

As for the moving of buffalo, we are simply identifying individuals that are eligible for the study so that when bison are released from the holding pens, they can be kept back for the study. We are moving bison to Brogan's now as a favor/IBMP partnering to NPS to hold bison until greenup. But study animals could be held back from that group.....therefore, they are already there...but have not been "transferred" on a research permit to us yet. Clear as mud? Becky

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425
(b) (6) cell
□ Pauline Nol/CO/APHIS/USDA

**Pauline
Nol/CO/APHIS/USDA**

ToRebecca K Frey/MT/APHIS/USDA@USDA
cc

Subjectplot gets thicker.....

03/09/2011 02:47 PM

Hey Becky,

I talked to Jenny Powers and she sent me the latest comments from her and Margaret Wild. This is getting way complicated actually so I don't see a realistic finalization of this for a while.

Both Margaret and Jenny agreed that having the third sterilized group would be really good. In fact, a better alternative to spayed animals would be tubally ligated animals. Then they would at least have all of their parts and be comparable to the other groups. The only difference is that they will be more likely to actually have sex, as opposed to the GnRH animals.

They were very concerned about the numbers being used, and I have to agree, since the magic number 10 isn't all that magical and we need to have more substance behind our choice of

sample size. I am working on that but probably need help on that. The one main thing is that I don't know what our space and financial limits are. Would you be able to give a fixed limit on numbers of animals we can have in the pastures and what we can afford to feed? I don't even know how we are paying for this study.

Margaret and Jenny suggested bringing Brandt Schumacher into the picture to help with an educated sample size generation, as well as analysis of the data ultimately. Brandt's with U WY now, and he's a good guy so it sound fine by me, but I'm sure recruiting him wouldn't be as simple as that.

There are many comments on wanting to carry the animals out further than what the protocol now suggests, which would be great to evaluate the vaccine, but don't know how financially or logistically feasible this would be. Maybe NPS could chip in if they really want to get those data points???

Lastly, I left a message with Rick Wallen to call me. Jenny was frankly surprised to hear that animals were being moved around at this point and so I was wanting to find out Rick's take on how urgent this protocol is to push through in relation to being able to keep these animals. You may have already told me this but, as I said, give me 15 minutes, and it's amazing what I forget.

This has the potential of being a very good, but much more complicated study than it started out as. I feel that if we are going to put the effort into it, we should learn as much as we can, and answer NPS's questions, since they are the people we are trying to convince to use the vaccine, if it looks feasible. But, this can become a huge monster too.

I have to go pick up kiddies now but I will work some more on this tomorrow.
Pauline

[attachment "Rhyon Immunocontraception Study Plan_rlw review brmd.doc" deleted by Rebecca K Frey/MT/APHIS/USDA]

From: [Rhyan, Jack C - APHIS](#)
To: [Miller - DNR, Mike](#); [Justin Scharton](#)
Cc: [McCollum, Matthew P - APHIS](#); [Barfield, JENNIFER](#); [Daylan Figgs](#); [Wolfe, Lisa \(Lisa.Wolfe@state.co.us\)](#); [Nol, Pauline - APHIS](#)
Subject: RE: Potential for new bison facility
Date: Wednesday, June 17, 2015 4:00:29 PM

Me too.

From: Miller - DNR, Mike [mailto:mike.miller@state.co.us]
Sent: Wednesday, June 17, 2015 12:30 PM
To: Justin Scharton
Cc: McCollum, Matthew P - APHIS; Barfield, JENNIFER; Daylan Figgs; Wolfe, Lisa (Lisa.Wolfe@state.co.us); Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: Re: Potential for new bison facility

Either is fine.

On Wed, Jun 17, 2015 at 12:14 PM, Justin Scharton <jscharton@fcgov.com> wrote:
Hi everyone –

Daylan and I are currently available on 7/1 after 11:00 AM to meet. Does an 11:00 or 1:00 meeting work for folks?

Thanks,

Justin

Justin W. Scharton

Environmental Planner | Land Management
City of Fort Collins Natural Areas Department
1745 Hoffman Mill Rd.
PO Box 580
Ft. Collins, CO 80524

[\(970\)221-6213](tel:(970)221-6213) Direct
<http://www.fcgov.com/naturalareas>



From: McCollum, Matthew P - APHIS [mailto:Matt.McCollum@aphis.usda.gov]
Sent: Thursday, June 04, 2015 2:56 PM
To: Miller - DNR, Mike

Cc: Barfield,JENNIFER; Daylan Figgs; Wolfe, Lisa (Lisa.Wolfe@state.co.us); Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Justin Scharton
Subject: RE: Potential for new bison facility

Thanks Mike et al,

I'm out of the office through the 15th of June and Jen is out the 10th of June through the 29th. Can we set a date shortly thereafter to get together and talk? How about Wednesday July 1 at your place? If that works for everyone, let's go with it. Otherwise, we could set up a doodle poll. Also, please include anyone who needs inclusion.

Oh give me a home...

Matt

From: Miller - DNR, Mike [<mailto:mike.miller@state.co.us>]
Sent: Thursday, June 04, 2015 1:03 PM
To: McCollum, Matthew P - APHIS
Cc: Barfield,JENNIFER; Daylan Figgs (dfiggs@fcgov.com); Wolfe, Lisa (Lisa.Wolfe@state.co.us); Rhyan, Jack C - APHIS; Nol, Pauline - APHIS; Justin Scharton (jscharton@fcgov.com)
Subject: Re: Potential for new bison facility

Thanks for bringing us all together, Matt.

We'd be happy to visit about this & will offer the conference area at our foothills facility for meeting if so desired. (We can open the door for a view of the prospective pasture area.)

As a point of clarification, the university grazed cattle in the area north of our facility off & on right up until the property was traded to the City (& maybe even for a bit after). I don't recall our ever having or asking for any restrictions on grazing activity, so I'm not sure what that's about. (We did need to "encourage" the cattle to stay away from our north perimeter from time to time, but that was out of concern for keeping their respiratory pathogens away from our bighorn sheep.)

Please let us know when you'd like to get together.

Cheers,

Mike

On Thu, Jun 4, 2015 at 11:56 AM, McCollum, Matthew P - APHIS

<Matt.McCollum@aphis.usda.gov> wrote:

Jen, Mike, and Daylan,

Lisa Wolfe was over this morning for some bison work and I talked to her about the idea of putting bison on the ARBL facility and City of Fort Collins land adjacent to the DOW wildlife facility. It sounds like everyone involved is supportive of the idea. This email is to put you all together to talk about the potential and what the sidebars are (see below for a little background). I've attached some maps/aerial photos that show where we are talking about.

Best,

Matt

From: Barfield,JENNIFER [mailto:[\(b\) \(6\)@colostate.edu](mailto:(b) (6)@colostate.edu)]

Sent: Tuesday, June 02, 2015 1:54 PM

To: McCollum, Matthew P - APHIS

Subject: new bison working facility

Matt,

I talked with Tod about using the LaPorte facility. He is supportive. I then talked with Jason Bruemmer who is managing that area for ARBL. He is also supportive. However, he did say that they weren't allowed to have cattle or ungulates in general up there because of the chronic wasting disease. This is something that DOW had mandated from their work right next to that area.

Also, checked on 50 this morning and she is definitely swollen!

Thanks!

Jen

Jennifer Barfield, PhD
Colorado State University
Department of Biomedical Sciences
1683 Campus Delivery
Fort Collins, CO 80523-1683

office: [\(970\) 491-8934](tel:(970)491-8934)

mobile: [\(b\) \(6\)](tel:(b) (6))

fax: [\(970\) 491-3557](tel:(970)491-3557)

Office: ARBL E101A, Foothills campus

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#)
Subject: Re: PS....you have her 1st calf. 3R88
Date: Tuesday, January 27, 2015 4:18:28 PM

???? I will look for the 1-27 but I think she left with 3R88

Becky
USDA APHIS VS
Sent from my iPhone

On Jan 27, 2015, at 4:15 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Could that calf be the girl we renamed 420? I don't have an 88 in the animals we ran through today.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, January 27, 2015 3:01 PM
To: Nol, Pauline - APHIS
Subject: PS....you have her 1st calf. 3R88

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [McCollum, Matthew P - APHIS](#)
Subject: Re: PS....you have her 1st calf. 3R88
Date: Tuesday, January 27, 2015 4:23:34 PM

Yep. 420. EID is334855

Becky
USDA APHIS VS
Sent from my iPhone

On Jan 27, 2015, at 4:19 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Ohhh ok!

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, January 27, 2015 4:18 PM
To: Nol, Pauline - APHIS
Cc: McCollum, Matthew P - APHIS
Subject: Re: PS....you have her 1st calf. 3R88

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From: Frey, Rebecca K - APHIS
Sent: Tuesday, January 27, 2015 3:01 PM
To: Nol, Pauline - APHIS
Subject: PS....you have her 1st calf. 3R88

From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: RE: PSPB results on GonaCon Study
Date: Monday, December 09, 2013 11:18:00 AM

Yeah, not bad! How's it going out there?

From: McCollum, Matthew P - APHIS
Sent: Monday, December 09, 2013 11:14 AM
To: Rhyan, Jack C - APHIS
Cc: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; Eckery, Douglas C - APHIS
Subject: Re: PSPB results on GonaCon Study
These are the sand dunes bison. Looks pretty good!

Sent from my iPhone

On Dec 9, 2013, at 11:07 AM, "Rhyan, Jack C - APHIS" <Jack.C.Rhyan@aphis.usda.gov> wrote:

From: BioTracking Testing Lab [<mailto:testinglab@biotracking.com>]
Sent: Friday, December 06, 2013 3:47 PM
To: Rhyan, Jack C - APHIS
Subject: BioPRYN Report for Bison & Buffalo

Dear BioPRYN Customer,

Here is the report on the samples we received from you. It's attached as an HTML file, and you should be able to directly open the attachment by double-clicking on it.

For up-to-date schedule and pricing information please visit our website at www.biotracking.com and click on Lab Services. Holiday closures and schedules are posted under Lab Services as well. We recommend checking the schedule prior to shipping samples to ensure you will receive results when expected. If you have any questions regarding the schedule, please do not hesitate to call and ask.

As always, we stand by our products and services, so please contact us here with any questions or comments you may have.

Thank you,

Amber Merk

Director of Laboratory Service & Sales

BioTracking LLC

1150 Alturas Dr. Ste. 105

Moscow, ID 83843

Office: 208.882.9736

Cell: (b) (6)

amerk@biotracking.com or testinglab@biotracking.com

Follow us on Facebook and Twitter

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: RE: PSPB results on GonaCon Study
Date: Monday, December 09, 2013 11:09:00 AM

These all Sanddunes?

From: Rhyan, Jack C - APHIS
Sent: Monday, December 09, 2013 11:07 AM
To: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; Eckery, Douglas C - APHIS
Subject: PSPB results on GonaCon Study

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Sent: Friday, December 06, 2013 3:47 PM

To: Rhyan, Jack C - APHIS

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As always, we stand by our products and services, so please contact us here with any questions or comments you may have.

Thank you,

Amber Merk

Director of Laboratory Service & Sales

BioTracking LLC

1150 Alturas Dr. Ste. 105

Moscow, ID 83843

Office: 208.882.9736

Cell: (b) (6)

amerk@biotracking.com or testinglab@biotracking.com

Follow us on Facebook and Twitter

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Eckery, Douglas C - APHIS](#)
Subject: Re: PSPB results on GonaCon Study
Date: Monday, December 09, 2013 11:13:47 AM

These are the sand dunes bison. Looks pretty good!

Sent from my iPhone

On Dec 9, 2013, at 11:07 AM, "Rhyan, Jack C - APHIS" <Jack.C.Rhyan@aphis.usda.gov> wrote:

From: BioTracking Testing Lab [<mailto:testinglab@biotracking.com>]

Sent: Friday, December 06, 2013 3:47 PM

To: Rhyan, Jack C - APHIS

Subject: BioPRYN Report for Bison & Buffalo

Dear BioPRYN Customer,

Here is the report on the samples we received from you. It's attached as an HTML file, and you should be able to directly open the attachment by double-clicking on it.

For up-to-date schedule and pricing information please visit our website at www.biotracking.com and click on Lab Services. Holiday closures and schedules are posted under Lab Services as well. We recommend checking the schedule prior to shipping samples to ensure you will receive results when expected. If you have any questions regarding the schedule, please do not hesitate to call and ask.

As always, we stand by our products and services, so please contact us here with any questions or comments you may have.

Thank you,

Amber Merk

Director of Laboratory Service & Sales

BioTracking LLC

1150 Alturas Dr. Ste. 105

Moscow, ID 83843

Office: 208.882.9736

Cell: (b) (6)

amerk@biotracking.com or testinglab@biotracking.com

Follow us on Facebook and Twitter

From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#)
Subject: RE: PSPB results on GonaCon Study
Date: Monday, December 09, 2013 12:37:00 PM

Good!

Is 25 gonna hang in your office?

From: McCollum, Matthew P - APHIS
Sent: Monday, December 09, 2013 12:05 PM
To: Nol, Pauline - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: Re: PSPB results on GonaCon Study
All done. Just waiting for 25's skull. Everything went well.

Sent from my iPhone

On Dec 9, 2013, at 11:18 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Yeah, not bad! How's it going out there?

From: McCollum, Matthew P - APHIS
Sent: Monday, December 09, 2013 11:14 AM
To: Rhyan, Jack C - APHIS
Cc: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS; Eckery, Douglas C - APHIS
Subject: Re: PSPB results on GonaCon Study
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To: Rhyan, Jack C - APHIS

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amerik@biotracking.com or testinglab@biotracking.com

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From: [Greiner, Steven J - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Greiner, Laura B - APHIS](#)
Subject: RE: QA 1858
Date: Tuesday, February 21, 2012 8:23:02 AM

Hi Pauline,

I was out of the office last week, so I'm trying to go through my emails and get caught up. In looking at your QA-1858, since it was reviewed and approved by the Bison Quarantine Facility's IACUC then I will defer the NWRC IACUC approval over to them. However, in your protocol you said that their IACUC approval letter is attached, but I did not see it. If you can send me a copy of their approval letter then I will give you the NWRC IACUC deferred approval. Thanks!

N. IACUC Approval

Date of IACUC Approval Letter: __ACUC Protocol approved 5/17/2011_See attached____

Bison Quarantine Facility Institutional Animal Care and Use Committee

Steve Greiner
Chair, NWRC IACUC
USDA/APHIS/WS National Wildlife Research Center
4101 LaPorte Ave, Fort Collins, CO 80521
Phone (970) 266-6169
Fax (970) 266-6010
steven.j.greiner@aphis.usda.gov

From: Nol, Pauline - APHIS
Sent: Thursday, February 16, 2012 10:53 AM
To: Greiner, Steven J - APHIS; Bens, Catherine M - APHIS; Greiner, Laura B - APHIS
Subject: QA 1858



Good morning!

I was wondering if you knew what the status was on this protocol: QA 1858 **Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Steven J - APHIS](#); [Greiner, Laura B - APHIS](#)
Subject: RE: QA 1858
Date: Tuesday, February 21, 2012 12:12:00 PM
Attachments: [ACUCBisonGonaConStudyfinal \(2\)with sigs.pdf](#)

Attached is the ACUC document.

Thanks!

From: Greiner, Steven J - APHIS
Sent: Tuesday, February 21, 2012 8:23 AM
To: Nol, Pauline - APHIS; Greiner, Laura B - APHIS
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GonaCon , an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan

Final ACUC protocol
5/23/11

REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. ____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011____ _____ Permit(s) description _____ Number _____ Date _____

DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
- ☒ *Manuscript/review article collaboration*
- ☐ *Training program requiring the use of animals*
- ☒ *Data analysis, interpretation and reporting*
- ☒ *Other: ___Live animal work___*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL**1. Key Personnel**

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator

Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October 1, 2019

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% ~~abortion~~ ^{shedding}). Two replicates of the two pastures will be conducted.

11. Animal Care and Use Information

- 1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 96 females, 8 males
Body weight range: 400-1000 kg
Age: 2 year to adult
- 2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.
- 3) Rationale for appropriateness of the species to be used: Bison are the target species.
- 4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.
- 6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.
- 8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart
Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Attn: Jack K Ryan

Page 9 of 9

Study Protocol

GonaCon-in-bison

PART ONE: SIGNATURE PAGE

Study Director: [Signature]

Date: 5/16/11

Concur: IACUC Chair [Signature] Date 5/16/11

From: [Wehtje, Morgan E - APHIS](#)
To: [Mora, Darcy - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Cc: [Eckery, Douglas C - APHIS](#)
Subject: RE: QA-1858 MT Bison GonaCon Study Questions
Date: Wednesday, November 16, 2016 4:27:35 PM

Hopefully Jack or Matt can help you. Pauline and I are in TX working on feral swine project planning. Have a good trip and I'll be interested to hear about what you found when you return.

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/VS/STAS/NVSL
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: Mora, Darcy - APHIS
Sent: Wednesday, November 16, 2016 2:52 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Wehtje, Morgan E - APHIS <Morgan.E.Wehtje@aphis.usda.gov>
Cc: Eckery, Douglas C - APHIS <Douglas.C.Eckery@aphis.usda.gov>
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Hi All,

We analyzed several hundred bison serum samples this week to determine anti-GnRH antibody levels. I am currently trying to make sense of this massive amount of data and wondering if one of you might be available at some point this afternoon to answer a couple of quick questions about the study design. I will be out of the office beginning tomorrow, returning on 11/28, but I would love to share this data before heading out if possible. Please let me know if anyone has a few minutes available today!

Thank you,

Darcy Orahoud Mora
Biologist
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone (970) 266-6061

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To: [Mora, Darcy - APHIS](#); [Rhyen, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
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Subject: Re: QA-1858 MT Bison GonaCon Study Questions
Date: Wednesday, November 16, 2016 4:34:20 PM

Shoot, sorry Darcy. I'm already home. Maybe you can catch Jack?

Heal fast!!!!

Matt

Sent from my iPhone

On Nov 16, 2016, at 2:51 PM, Mora, Darcy - APHIS <Darcy.Mora@aphis.usda.gov> wrote:

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Cc: [Eckery, Douglas C - APHIS](#)
Subject: RE: QA-1858 MT Bison GonaCon Study Questions
Date: Wednesday, November 16, 2016 4:34:19 PM

Thanks Morgan! Jack was able to address the questions... but those answers led to the conclusion that we are seeing some strange results in the control animals at a couple of time points so we'll do some troubleshooting with the assay in December.

Good luck to you and Pauline in TX.

Regards,

Darcy Orahood Mora
Biologist
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone (970) 266-6061

From: Wehtje, Morgan E - APHIS
Sent: Wednesday, November 16, 2016 4:28 PM
To: Mora, Darcy - APHIS <Darcy.Mora@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>
Cc: Eckery, Douglas C - APHIS <Douglas.C.Eckery@aphis.usda.gov>
Subject: RE: QA-1858 MT Bison GonaCon Study Questions

Hopefully Jack or Matt can help you. Pauline and I are in TX working on feral swine project planning. Have a good trip and I'll be interested to hear about what you found when you return.

Morgan Wehtje, Wildlife Biologist
Wildlife Livestock Disease Investigations Team
USDA/APHIS/Vs/STAS/NVSL
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, Colorado, USA 80521
Phone: +1 970 266 6318
Fax: +1 970 266 6157
Email: Morgan.E.Wehtje@aphis.usda.gov

From: Mora, Darcy - APHIS
Sent: Wednesday, November 16, 2016 2:52 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>;

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Cc: Eckery, Douglas C - APHIS <Douglas.C.Eckery@aphis.usda.gov>
Subject: QA-1858 MT Bison GonaCon Study Questions

Hi All,

We analyzed several hundred bison serum samples this week to determine anti-GnRH antibody levels. I am currently trying to make sense of this massive amount of data and wondering if one of you might be available at some point this afternoon to answer a couple of quick questions about the study design. I will be out of the office beginning tomorrow, returning on 11/28, but I would love to share this data before heading out if possible. Please let me know if anyone has a few minutes available today!

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From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: RE: Question re July 2014 bull collections in MT
Date: Monday, January 12, 2015 7:33:18 AM

I have a record from my stock on July 17th for a 3YO bull. Which is when you all were here to collect bulls...so it looks like mine.

From: McCollum, Matthew P - APHIS
Sent: Tuesday, January 06, 2015 9:34 AM
To: Frey, Rebecca K - APHIS
Subject: FW: Question re July 2014 bull collections in MT

Hiya,

Do you recall?

From: Nol, Pauline - APHIS
Sent: Tuesday, January 06, 2015 9:22 AM
To: McCollum, Matthew P - APHIS
Subject: RE: Question re July 2014 bull collections in MT

I can't find any records, do you think Becky might have recorded it?

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
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National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: McCollum, Matthew P - APHIS
Sent: Wednesday, December 31, 2014 3:48 PM
To: Nol, Pauline - APHIS
Subject: Re: Question re July 2014 bull collections in MT

Pretty sure it was ours.

Sent from my iPhone

On Dec 31, 2014, at 12:14 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

I think you said you had to tip some over completely. Did we use our A3080 or Becky's?
Thanks!

Pauline Nol, DVM, MS, PhD
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From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: Question re July 2014 bull collections in MT
Date: Wednesday, December 31, 2014 3:47:45 PM

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Sent from my iPhone

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From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: Questions on attached paperwork
Date: Thursday, January 16, 2014 10:53:02 AM

PO Box. I have a few sheets that I think are bottles that Matt brought with him and we used up, but there are no numbers. Let me look one other place....I might have some more if I faxed them.

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Thursday, January 16, 2014 10:28 AM
To: Frey, Rebecca K - APHIS
Subject: RE: Questions on attached paperwork

I thought maybe you had the originals because I only have fax/copies of the 2008 stuff. The pdf has the sheets that are missing friends. Kind of like socks!

Is your mailing address for your (b) (6) you street address or the PO box?

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Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Thursday, January 16, 2014 10:26 AM
To: Nol, Pauline - APHIS
Subject: Re: Questions on attached paperwork

Uh oh. I don't know if I can help you cuz I have given you all of my sheets through last year. I have the immobilization charts that shows each animal I darted..... But the records for each bottle have all gone back to you. Yikes!

Becky
USDA APHIS VS
Sent from my iPhone

On Jan 16, 2014, at 10:21 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Hi Becky!

I have to start submitting A3080 paperwork to WildPharm and am going to send one year at a time. I'm missing immob. sheets or reversal sheets for some of the 2008 work and I don't have a complete drug log for bottle 08-3. Could you help me with this when you get a chance? I expect that to be February, but that's okay.

I'm sure I'll be sending similar emails for the subsequent years too.

Thanks!

Pauline

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<2008 Immobilization Paperwork for Becky.pdf>

From: [Herriott, Donald E - APHIS](#)
To: [Ahola, Sara C - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Re: research planning meeting
Date: Thursday, November 17, 2016 1:56:07 PM

Well I have a huddle room with white board for an office if the group is not too big.

Don

Sent from my iPad

On Nov 17, 2016, at 1:51 PM, "Ahola, Sara C - APHIS" <Sara.C.Ahola@aphis.usda.gov> wrote:

Count me in. I recommend NWRC – Bldg B is getting new paint/carpet and many of the meeting rooms are unavailable.

~Sara

From: Clarke, Patrick R. - APHIS
Sent: Thursday, November 17, 2016 1:48 PM
To: Herriott, Donald E - APHIS <Don.E.Herriott@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>; Ahola, Sara C - APHIS <Sara.C.Ahola@aphis.usda.gov>
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Ryan

P. Ryan Clarke, DVM, MPH

USDA, APHIS, VS, SPRS
District 5 Epidemiologist-GYA
Bozeman, Montana
406-388-5162

From: [Ahola, Sara C - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
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From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: sample list
Date: Tuesday, January 27, 2015 4:17:16 PM

She is a seropositive, given GonaCon last May. Since she was pregnant, you took her so we had another 100% contracepted group. We didn't have a place for her since our control group had not been bred yet.

Becky
USDA APHIS VS
Sent from my iPhone

On Jan 27, 2015, at 4:13 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

So this girl was negative this go around, right? I can't find her serology history in my records. Curious as to what it looks like.

She's the only pregnant one in the bunch we brought back??

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Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, January 27, 2015 3:10 PM
To: Nol, Pauline - APHIS
Subject: sample list

Hi,

So when the Red 52 calves.....and her implant is 151.134.....we are taking blood, serum and whole; feces; vaginal swab; any fluid or placenta; milk, all four quarters into one tube; conjunctival swab, and blood from calf.....or submit the abortion. All within 5 days of birth. Usually within 3 days though.....The swabs go into 1 ml WHO. All samples need quantification from NVSL. I don't know how long you will keep her, but those are the samples we will need to compare her to the rest of the group one day.

Thanks!

Becky

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: sample list
Date: Tuesday, January 27, 2015 4:18:00 PM

But weren't they all negative this time around according to the Montana lab?

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Becky

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: sample list
Date: Tuesday, January 27, 2015 4:19:58 PM

No. Only the calves you took this year had a result in. I don't have any results from MT yet on the rest. She should be positive.

Becky
USDA APHIS VS
Sent from my iPhone

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To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Held, Karl E - APHIS](#)
Subject: RE: scan
Date: Friday, February 06, 2015 1:56:13 PM

We should move 421 into a b+ pen soon.

-----Original Message-----

From: Frey, Rebecca K - APHIS
Sent: Friday, February 06, 2015 11:51 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: scan

July test of Red 421.....see 81AJW3758

Wildlife Disease Specialist
Veterinary Services
Montana

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Friday, July 25, 2014 3:35 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

From: [Rhyan, Jack C - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: scan
Date: Wednesday, June 18, 2014 10:47:19 AM
Attachments: [Strep uberis abortion in cattle abst.docx](#)

Hmmm. I always hate to let a lab report mess up a good diagnosis. Anyway, see the attached abstract. My thought is that bruc had a role but maybe later was overgrown with the Strep. All we can say is "Bruc not cultured, Strep cultured."

Jack

-----Original Message-----

From: Clarke, Patrick R. - APHIS
Sent: Wednesday, June 18, 2014 8:38 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: FW: scan

Jack, what do you think?

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

-----Original Message-----

From: livdiagnosticlab@mt.gov [<mailto:livdiagnosticlab@mt.gov>]
Sent: Monday, June 16, 2014 4:22 PM
To: Clarke, Patrick R. - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

Title

Association of *pasteurella haemolytica* with bovine abortion - a case report.

Authors

Rajesh Chahota; Katoch, R. C.; Arvind Mahajan; Subhash Verma

Author Affiliation

Department of Veterinary Microbiology, Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh - 176062, India.

Journal

Indian Veterinary Journal 2000 Vol. 77 No. 9 pp. 807-808

ISSN

0019-6479

Record Number

20003023400

[Email this record](#)

Abstract

The necropsy examination of the fetus of a bovine that had a sudden commencement of abortion at 6 months of pregnancy is presented [Himachal Pradesh, India; date not given]. Serosanguinous fluid was observed in the peritoneal, thoracic and pericardial cavities, while the myocardium was severely congested. *P. haemolytica* was isolated from peritoneal and pericardial fluids, heart, blood and spleen, while *Streptococcus uberis* was isolated from the peritoneal and pericardial fluids, heart, blood, spleen and stomach contents. *S. uberis*, which is a normal inhabitant of vaginal tract and bovine mastitis, may have gained access to the fetus via relaxed cervix or when the fetus would have passed through the vaginal tract

From: [Held, Karl E - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: scan
Date: Friday, February 13, 2015 6:02:02 PM

421 is one of the little heiffers. Maybe 2ish? She was a bit gimpy when she arrived. She has a red tag and I think she is last in the pecking order.

Sent from my iPhone

> On Feb 13, 2015, at 1:38 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

>

> Is she bred?

>

>

> -----Original Message-----

> From: McCollum, Matthew P - APHIS

> Sent: Friday, February 06, 2015 1:56 PM

> To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; Held, Karl E - APHIS

> Subject: RE: scan

>

> We should move 421 into a b+ pen soon.

>

> -----Original Message-----

> From: Frey, Rebecca K - APHIS

> Sent: Friday, February 06, 2015 11:51 AM

> To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

> Subject: FW: scan

>

> July test of Red 421.....see 81AJW3758

>

> Wildlife Disease Specialist

> Veterinary Services

> Montana

>

>

> -----Original Message-----

> From: livdiagnosticlabb@mt.gov [<mailto:livdiagnosticlabb@mt.gov>]

> Sent: Friday, July 25, 2014 3:35 PM

> To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS

> Subject: scan

>

> -----

> KM-2560

> [00:c0:ee:1e:d7:d6]

> -----

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#); [Held, Karl E - APHIS](#)
Subject: RE: scan
Date: Friday, February 13, 2015 3:52:17 PM

no

-----Original Message-----

From: Nol, Pauline - APHIS
Sent: Friday, February 13, 2015 1:38 PM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS; Held, Karl E - APHIS
Subject: RE: scan

Is she bred?

-----Original Message-----

From: McCollum, Matthew P - APHIS
Sent: Friday, February 06, 2015 1:56 PM
To: Nol, Pauline - APHIS; Rhyan, Jack C - APHIS; Held, Karl E - APHIS
Subject: RE: scan

We should move 421 into a b+ pen soon.

-----Original Message-----

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Sent: Friday, February 06, 2015 11:51 AM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: FW: scan

July test of Red 421.....see 81AJW3758

Wildlife Disease Specialist
Veterinary Services
Montana

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Sent: Friday, July 25, 2014 3:35 PM
To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: scan

KM-2560
[00:c0:ee:1e:d7:d6]

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: sera
Date: Tuesday, November 19, 2013 10:13:47 AM

[Me too.](#)

From: Nol, Pauline - APHIS
Sent: Monday, November 18, 2013 5:29 PM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS
Subject: RE: sera

[I do not.](#)

From: McCollum, Matthew P - APHIS
Sent: Monday, November 18, 2013 3:08 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: sera

[Either of yous two know?](#)

[M](#)

From: Frey, Rebecca K - APHIS
Sent: Monday, November 18, 2013 2:05 PM
To: McCollum, Matthew P - APHIS
Subject: sera

Hey, Did Ryan send you any of the serum from the GonaCon cows for gonacon titers this summer? If so, did you get the results?

Thanks

Killed a cow and 3 does so far.....got meat?

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: RE: sera
Date: Monday, November 18, 2013 5:28:00 PM

I do not.

From: McCollum, Matthew P - APHIS
Sent: Monday, November 18, 2013 3:08 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: sera
[Either of yous two know?](#)

M

From: Frey, Rebecca K - APHIS
Sent: Monday, November 18, 2013 2:05 PM
To: McCollum, Matthew P - APHIS
Subject: sera

Hey, Did Ryan send you any of the serum from the GonaCon cows for gonacon titers this summer? If so, did you get the results?

Thanks

Killed a cow and 3 does so far.....got meat?

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: sera
Date: Tuesday, November 19, 2013 11:37:53 AM

Our records show we received it in August. Matt is checking the freezer for it.

Thanks.

Jack

From: Nol, Pauline - APHIS
Sent: Monday, November 18, 2013 5:29 PM
To: McCollum, Matthew P - APHIS; Rhyan, Jack C - APHIS
Subject: RE: sera
I do not.

From: McCollum, Matthew P - APHIS
Sent: Monday, November 18, 2013 3:08 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Subject: FW: sera
Either of yous two know?
M

From: Frey, Rebecca K - APHIS
Sent: Monday, November 18, 2013 2:05 PM
To: McCollum, Matthew P - APHIS
Subject: sera
Hey, Did Ryan send you any of the serum from the GonaCon cows for gonacon titers this summer? If so, did you get the results?
Thanks
Killed a cow and 3 does so far.....got meat?
Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Quance, Christine R \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#); [Robbe Austerman, Suelee \(APHIS\)](#); [Henry, Lisa A \(APHIS\)](#)
Cc: [Frey, Rebecca K \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#)
Subject: RE: seropositive brucella cows in Idaho
Date: Wednesday, August 17, 2011 8:18:56 AM

Hi Jack,

Thanks for sending this. We are registered with the CDC now instead of APHIS, but they should have the same rules.

I'll send a separate email on the media.

Chris Quance
Microbiologist-Team Leader, Mycobacteria and Brucella Section
National Veterinary Services Laboratory
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.R.Quance@aphis.usda.gov

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This communication, together with any attachments or links contained herein, is for the sole use of the intended recipient(s) and may contain information that is confidential or legally protected. If you have received this communication in error, please notify the sender immediately and destroy the document.

-----Original Message-----

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, August 16, 2011 5:30 PM
To: Quance, Christine R (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: FW: seropositive brucella cows in Idaho

Chris,

See below the info from Freeda that says we now can sample a naturally infected animal time after time and the specimen is the select agent once it is confirmed positive. We will be doing this both with some seropositive bison bulls soon and starting next spring with the contraception study. I'll give you a call and talk about the bull study. Also, who should I talk to about getting some fresh WHO media in tubes?

Jack

-----Original Message-----

From: Freeda E Isaac [<mailto:freeda.e.isaac@aphis.usda.gov>]
Sent: Thursday, May 13, 2010 11:50 AM
To: Jack C Rhyan
Subject: Fw: seropositive brucella cows in Idaho

Hi Jack,

As we had discussed over the phone several weeks ago, the Select Agent Program directors were meeting to discuss the issues related to naturally infected versus experimentally infected animals and the status of samples taken from these animals.

In our discussions, it was agreed upon that for naturally affected animals, samples taken from those animals would not be considered select agent material and required to be handled as restricted material until the sample was confirmed to have select agent material. For the issues you have raised below for the cattle you have, the samples may be handled as you have described and not subject to select agent requirements until the sample itself is

confirmed positive for select agents.

For your questions regarding registration with the select agent program, you would need to have a security risk assessment completed which is a background check by FBI. That would be different than what would have been done at Plum Island previous to about 2005. I will give you a call to discuss other issues with the cattle. For information on completing the registration documents, you can call Sherylyn Roberson at 301-734-5460.

Thanks, Freeda

Freeda E. Isaac, DVM
Director
National Center for Import Export
USDA/APHIS/Veterinary Services
Phone: 301-734-8364
Fax: 301-734-6402
Email: Freeda.E.Isaac@aphis.usda.gov

----- Forwarded by Freeda E Isaac/MD/APHIS/USDA on 05/13/2010 01:35 PM -----

Freeda E Isaac/MD/APHIS/USDA
04/02/2010 12:14 PM
To
Jack C Rhyan/CO/APHIS/USDA@USDA

cc
Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Matt
McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: seropositive brucella cows in Idaho

Hi Jack,

Although naturally infected animals are not considered select agents themselves and not subject to the select agent regulations, once these animals are confirmed as positive for a select agent, any materials from these animals would be treated as select agent material. The infected cattle are considered the natural source of the Brucella and the materials from these animals are being intentionally collected. This is found in 9 CFR 121.3(d)(1). For example, blood, tissue specimens, urine, etc. would be subject to handling as select agent material. My understanding is these Idaho cattle have been confirmed for B. abortus by VS, therefore the materials from these cattle would need to be handled in accordance with the select agent requirements.

9 CFR 121.6(a)(1) requires that specimens are transferred to a select agent registered facility for that particular select agent within 7 calendar days. 9 CFR 121.16 describes the transfer process in which a APHIS/CDC Form 2 is completed and submitted to APHIS for approval prior to the transfer.

Let me know if you have any other questions. Freeda

Freeda E. Isaac, DVM

Director
National Center for Import Export
USDA/APHIS/Veterinary Services
Phone: 301-734-8364
Fax: 301-734-6402
Email: Freeda.E.Isaac@aphis.usda.gov

Jack C Rhyan/CO/APHIS/USDA
03/22/2010 05:44 PM
To
Freeda E Isaac/MD/APHIS/USDA@USDA

cc
Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Pauline
Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA

Subject
seropositive brucella cows in Idaho

Freeda,
Current situation: Idaho has 4 seroconverters, at least 2 have aborted. Milk and fetus from one cow has been submitted for culture.
My proposal: I would purchase the 4 and bring to Fort Collins (with state vet's and AVIC's approval). We would then collect large amounts of blood for NVSL's serum bank. We would periodically collect urine from them; filter it to exclude bacteria and submit urine samples for GC/mass spec analysis for volatile organic compounds (VOCs) specific for brucella. We would also analyze breath for VOCs. Breath analysis and urine can be done here. Urine also might be shipped to APHIS personnel at a laboratory in PA for GC/MS analysis. I think this project would last 6 months at the end of which we would kill cows and submit specimens for culture.

Please advise me on this potential study in view of select agent requirements.
Thanks much for your help.
Jack

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 11:24:00 AM

Sheesh! And I guess one of them still hasn't made it...

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 11:21 AM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
Weird....NVSL got theirs yesterday

|

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 10:36 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
Looks like we just gottem☺
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 10:27 AM
To: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
NVSL number is 7730 9132 1245
I did not give Chris a heads up....will check with her now.
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:10 AM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS

Subject: RE: serum is shipped. 3 boxes

Still in transit. Ryan, what is the NVSL number? Did you let Chris know is was coming?

Rebecca Frey

Wildlife Disease Specialist

Veterinary Services

Montana

From: Clarke, Patrick R. - APHIS

Sent: Thursday, March 12, 2015 10:07 AM

To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS

Subject: RE: serum is shipped. 3 boxes

yep

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA, APHIS, VS, District 5

406-388-5162

From: Nol, Pauline - APHIS

Sent: Thursday, March 12, 2015 10:07 AM

To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS

Subject: RE: serum is shipped. 3 boxes

Fed Ex right?

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

Office: 970-266-6126

Cell: (b) (6)

Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS

Sent: Thursday, March 12, 2015 10:06 AM

To: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS

Subject: RE: serum is shipped. 3 boxes

Tracking number is 7730 9154 5145

Should have arrived yesterday afternoon

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA, APHIS, VS, District 5

406-388-5162

From: Frey, Rebecca K - APHIS

Sent: Thursday, March 12, 2015 9:55 AM

To: Clarke, Patrick R. - APHIS

Subject: FW: serum is shipped. 3 boxes

From: Nol, Pauline - APHIS

Sent: Thursday, March 12, 2015 9:55 AM

To: Frey, Rebecca K - APHIS

Subject: RE: serum is shipped. 3 boxes

Becky,

What's the tracking number? Haven't gotten the samples yet.

P

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS

Sent: Tuesday, March 10, 2015 2:43 PM

To: Nol, Pauline - APHIS

Subject: serum is shipped. 3 boxes

The GonaCon titer bag of serum is in one of the boxes. I am faxing the list of serum/ID's in the bag....after I get a new phone cord ☺ The rest are small boxes of 1st cohort BQFS serum. We ran out of boxes for the tall serum tubes, but I think that you now have all of the 1st cohort serum. It is labeled kinda of every which way.....because 3 different people stored it....over the years. Lucky for us....I have all of the original test charts, and the database in order to figure out what is what, as many are labeled by case number of the lab. They are all labeled for 1st cohort and a date though. We still have to finish boxing up the 2nd cohort, and when we get done will know how much more room we need to create in our freezer.....and may need to ship down some more to you. It doesn't hardly look like we did anything today!!!

Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 10:36:00 AM

Looks like we just gottem☺

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
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P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:10 AM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
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Still in transit. Ryan, what is the NVSL number? Did you let Chris know is was coming?
Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 10:07 AM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
yep
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 10:07 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
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Fed Ex right?
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS

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Office: 970-266-6126
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To: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
Tracking number is 7730 9154 5145
Should have arrived yesterday afternoon
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 9:55 AM
To: Clarke, Patrick R. - APHIS
Subject: FW: serum is shipped. 3 boxes

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Sent: Thursday, March 12, 2015 9:55 AM
To: Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
Becky,
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P

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Becky
Rebecca Frey
Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 10:13:00 AM

Since it's all serum and it's not summertime, it'll be alright.

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:11 AM
To: Nol, Pauline - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: serum is shipped. 3 boxes
Somewhere between Memphis and FC.
Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 10:11 AM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: serum is shipped. 3 boxes
Where is it stuck?

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:10 AM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
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Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Clarke, Patrick R. - APHIS
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Subject: RE: serum is shipped. 3 boxes
yep
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

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Sent: Thursday, March 12, 2015 10:07 AM
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Subject: RE: serum is shipped. 3 boxes
Fed Ex right?
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)

Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 10:06 AM
To: Frey, Rebecca K - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
Tracking number is 7730 9154 5145
Should have arrived yesterday afternoon
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 9:55 AM
To: Clarke, Patrick R. - APHIS
Subject: FW: serum is shipped. 3 boxes

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 9:55 AM
To: Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes

Becky,

What's the tracking number? Haven't gotten the samples yet.

P

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, March 10, 2015 2:43 PM
To: Nol, Pauline - APHIS
Subject: serum is shipped. 3 boxes

The GonaCon titer bag of serum is in one of the boxes. I am faxing the list of serum/ID's in the bag....after I get a new phone cord ☺ The rest are small boxes of 1st cohort BQFS serum. We ran out of boxes for the tall serum tubes, but I think that you now have all of the 1st cohort serum. It is labeled kinda of every which way.....because 3 different people stored it....over the years. Lucky for us....I have all of the original test charts, and the database in order to figure out what is what, as many are labeled by case number of the lab. They are all labeled for 1st cohort and a date though. We still have to finish boxing up the 2nd cohort, and when we get done will know how much more room we need to create in our freezer.....and may need to ship down some more to you. It doesn't hardly look like we did anything today!!!

Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana
406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 10:10:00 AM

Where is it stuck?

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:10 AM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
Still in transit. Ryan, what is the NVSL number? Did you let Chris know it was coming?
Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 10:07 AM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
yep
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 10:07 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
Fed Ex right?
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
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Fort Collins, CO 80521
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Wildlife Livestock Disease Investigations Team

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4101 LaPorte Ave.

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Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 10:09:36 AM

Still in transit. Ryan, what is the NVSL number? Did you let Chris know it was coming?

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Clarke, Patrick R. - APHIS
Sent: Thursday, March 12, 2015 10:07 AM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes

yep

P. Ryan Clarke, DVM, MPH
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406-388-5162

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To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS
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Fed Ex right?

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Subject: RE: serum is shipped. 3 boxes

Tracking number is 7730 9154 5145

Should have arrived yesterday afternoon

P. Ryan Clarke, DVM, MPH
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Becky,

What's the tracking number? Haven't gotten the samples yet.

P

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Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Clarke, Patrick R. - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 10:06:16 AM

Tracking number is 7730 9154 5145
Should have arrived yesterday afternoon
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA, APHIS, VS, District 5
406-388-5162

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Subject: RE: serum is shipped. 3 boxes

Becky,

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Becky

Rebecca Frey

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Montana
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Date: Thursday, March 12, 2015 10:06:00 AM

Fed Ex right?

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
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Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Thursday, March 12, 2015 9:54:00 AM

Becky,

What's the tracking number? Haven't gotten the samples yet.

P

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
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Thanks

Becky

Rebecca Frey
Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Wednesday, March 11, 2015 9:23:00 AM

Got it! Thanks!

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Wednesday, March 11, 2015 8:58 AM
To: Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes

I think it went

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Wednesday, March 11, 2015 8:55 AM
To: Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
9702666157

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Wednesday, March 11, 2015 8:40 AM
To: Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes

I am trying to send you a fax. The number I have is 9702666138?

Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Tuesday, March 10, 2015 3:09 PM
To: Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes

Okay, I'll look for the package tomorrow. Isn't freezer diving fun??;)

From: Frey, Rebecca K - APHIS

Sent: Tuesday, March 10, 2015 2:43 PM

To: Nol, Pauline - APHIS

Subject: serum is shipped. 3 boxes

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Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

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9702666157

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Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: serum is shipped. 3 boxes
Date: Friday, March 13, 2015 12:51:00 PM

Finally got the third box!

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:11 AM
To: Nol, Pauline - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: serum is shipped. 3 boxes
Somewhere between Memphis and FC.
Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

From: Nol, Pauline - APHIS
Sent: Thursday, March 12, 2015 10:11 AM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: RE: serum is shipped. 3 boxes
Where is it stuck?

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 12, 2015 10:10 AM
To: Clarke, Patrick R. - APHIS; Nol, Pauline - APHIS
Subject: RE: serum is shipped. 3 boxes
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Rebecca Frey
Wildlife Disease Specialist
Veterinary Services
Montana

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Sent: Thursday, March 12, 2015 10:07 AM
To: Nol, Pauline - APHIS; Frey, Rebecca K - APHIS
Subject: RE: serum is shipped. 3 boxes
yep
P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
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406-388-5162

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Subject: RE: serum is shipped. 3 boxes
Fed Ex right?
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Becky,
What's the tracking number? Haven't gotten the samples yet.

P

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Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana
406-333-4425 office/fax

From: [Nol, Pauline \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Clarke, Patrick R. \(APHIS\)](#)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call
Date: Thursday, June 16, 2011 12:34:00 PM

Yes.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (b) (6)

From: Rhyan, Jack C (APHIS)
Sent: Thursday, June 16, 2011 12:17 PM
To: Nol, Pauline (APHIS); McCollum, Matthew P (APHIS); Frey, Rebecca K (APHIS); Clarke, Patrick R. (APHIS)
Subject: FW: Some Q's on the GonaCon protocol and request for conf call

Looks like this time may work for everyone in Riverdale. Can you all make it? It would be helpful.
Jack

From: Rhyan, Jack C (APHIS)
Sent: Thursday, June 16, 2011 10:27 AM
To: Edmundson, Jack P (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

How 'bout Tuesday the 21st at 3:30 eastern (1:30 in CO)?
Jack

From: Edmundson, Jack P (APHIS)
Sent: Tuesday, June 14, 2011 8:10 AM
To: Rhyan, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

I think Tuesday (6/21) after 1:30 (Eastern time) would be best. (Stephanie is out on 6/16 and 6/17, but will be back next week and she really should be there.) The next best day would be 6/20 (I won't be available, but Stephanie should be back and most of the others should be available then, also). –
Jack E.

From: Rhyan, Jack C (APHIS)
Sent: Monday, June 13, 2011 2:37 PM
To: Edmundson, Jack P (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: RE: Some Q's on the GonaCon protocol and request for conf call

Jack,

I started to answer these but decided it would be best to conf call if possible. I'm on travel; be back on Thursday. Can you all do a call Thursday or Friday?

Jack

From: Edmundson, Jack P (APHIS)
Sent: Friday, June 10, 2011 12:59 PM
To: Rhyan, Jack C (APHIS)
Cc: Gutierrez, Vicki L (APHIS); Stephens, Stephanie H (APHIS); Nasr, Ann M (APHIS); Willard, Tracy A (APHIS); Donch, Debra A (APHIS)
Subject: Some Q's on the GonaCon protocol and request for conf call

Hi, Jack. We pulled the Bison Team together the other day to begin work in earnest on the GonaCon EA. The first thing we did was go through the protocol with a fine-toothed comb to be sure we understood exactly what we are planning to do. Based on some things we have seen from BFC we suspect that they will be all over the study and watching like a hawk. As I understand it, the propocol you sent us is the final one that has been approved by NPS and a permit has been issued based on it. (In other words, APHIS shouldn't change anything in it because it would be a major paperwork hassle.) With that as background, we do have a few comments/questions about the protocol:

- How come we need a YNP permit to do work outside of the Park? And what exactly does the permit cover and not cover?

It is for the collection of bison at the trap at Stevens Creek.

- For NEPA purposes, is the lead agency APHIS or APHIS-VS? Will NPS (or NPS and APHIS-VS) officially be a cooperator in the EA? If NPS is an official cooperator, it could add additional review/approval time because NPS would have to be involved. Does NPS expect to be a NEPA Cooperator?

Concerning WS, I'll refer this question to Kathy with WS. I should visit with the Park about the EA. My guess is if they are official cooperators, it will add 3 to 10 years to the EA process.

- What is the relationship of the study to FIFRA Registration?

What is FIFRA?

- What are the roles of WS and NPS? Will they actually help in the field? Analyze info? Review/comment on things? The Park will likely work with us a little in the field. WS will also be involved a little bu primarily in the lab.
- The study says it starts on June 1, 2011, presumably because we collected animals after that? From a NEPA standpoint, we would prefer to have it start in 2012 when we begin to inject animals. We have already said that NEPA did not need to be done to collect animals for research. And, if we say it has already started, then technically NEPA should already be completed. (Also, for a 7 year study, it should end in 2019, not 2017.) You're right.
- Is Cammie Johnson our statistician? Should we list her in the investigators? We haven't involved Cammie on this one.

- The 3rd Objective does not seem to have a hypothesis associated with it. Also, the only thing in the Methods/Procedures section that could relate is the paragraph talking about what is to happen if there is an abortion in the field. It is not tied together very clearly (at least not enough for us to explain it to the public, as we must do in the EA).
- In several places we talk about marking animals, but it is not real clear how. For instance on p.4 #8 we mention collars, but elsewhere we talk about ear tags and microchips. We will need to talk about which methods we use and when.
- There is some confusion in our minds about the months when things happen. For instance, on page 5 we identify a time period when bulls will be separated from cows as outside the breeding season (from Oct to July), and the abortion/calving season from Feb to Aug. These dates will allow bulls to be with cows in August, when they could be exposed to abortions/birth-related shedding.
- We were confused by the statistics section and will probably need to be walked through that so that we can understand what we are measuring and what it means.
- There is also some confusion about when we can donate to food banks, when incineration will be used, when chemicals will be used for immobilization and/or euthanasia.

There are additional small points we would want to just talk with you about to get them straight in our minds or to ask your advice as to how to best present them in an EA. Can we organize a conference call with you to talk some of these things out? Since I am getting ready to retire, I'll be phasing out of the bison business (one of my regrets at retiring) and Stephanie Stephens will be taking my place. Since she (and Vicki) will be leading the NEPA effort, she will be getting in contact with you to set up the conference call, but we wanted you to have at least a partial list of the things we have been thinking about.

Jack E

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#)
Subject: RE: strategiic and work plans -see what you think
Date: Wednesday, October 29, 2014 4:29:00 PM
Attachments: [WiLDIT Strategic PlanFY2015 PN.docx](#)
[WiLDIT Work Plan FY2015 PN.docx](#)

Some edits just in case these get requested.

From: Rhyan, Jack C - APHIS
Sent: Wednesday, October 29, 2014 10:21 AM
To: Nol, Pauline - APHIS
Subject: strategiic and work plans -see what you think

WiLDIT Strategic Plan

October 28, 2014

The mission of WiLDIT is to develop science-based solutions to disease problems at the wildlife/domestic animal interface.

To accomplish this mission, WiLDIT engages in 4 separate areas of activity or “strategies.”

1. Consultation – provide information and advice to USDA/APHIS and other State and Federal agencies and research partners on interface disease issues; serve as a liaison with agencies, universities and NGOs.
2. Developmental work – Coordinate and/or conduct developmental work to address VS-specific interface disease problem areas, i.e. vaccines and delivery systems for use in wildlife; remote screening and diagnostic techniques for use in wild populations; and strategies to detect, manage, and eradicate diseases in wild populations. Developmental work is usually accomplished through collaborations with other agencies and universities.
3. Training and mentoring? – Serve as a training resource for the agency concerning interface diseases.
4. Monitoring/Surveillance – Conduct surveillance of wild populations around disease outbreaks or on a continuing basis in endemic areas when requested.

WiLDIT Work Plan FY2015

Feral Swine Work:

Continue pig TB and *Brucella* vaccine work. Specifically, obtain Hawaii pigs and start colony. Conduct Texas pig trial with killed *M. bovis*. Plan for next *Brucella* trial.

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Collect VOCs from pigs in experimental infections, Spanish wild boar, and any other field opportunities that arise.

Conduct oral fluid test to see which bait is most attractive. Collect oral fluids from pigs as opportunities arise.

Begin pathogenesis and vaccine work on FMD, CSF, and/or ASF.

Elk Work

Repeat *Brucella* natural exposure model experiment.

Continue work on spray-dried vaccine for *Brucella*

Explore surveillance work on or around Hardware ranch

Bison Work

Continue GonaCon studies in MT and at Great Sand Dunes

Compile data for John Eisemann to pursue getting GonaCon registered for bison-on-label

Continue Drydart development – small bison and elk study

Pursue GTNP and/or YNP brucellosis vaccination field trial

Continue to assist CSU on assisted reproductive techniques work

Other

Mouse studies evaluating spray-dried *Brucella* vaccines

Formatted: Font: Italic

Assist Torsten Eckstein with lipid ELISA development

Polar bear work

Ecology of Fish brucellosis

Various other collaborations beneficial to VS (as they arise)

From: [Quance, Christine R - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Cc: [Robbe Austerman, Suelee - APHIS](#)
Subject: RE: test results
Date: Wednesday, June 18, 2014 3:41:07 PM

Hi Rebecca,

I am copying others on this just so everyone is on the same page!!

You should have results from the February submissions... Acc. 14-004962 and 14-004963... these were GonaCon samples (vag swabs and blood).

Let me know if you need those reports resent.

Everything else is pending.

14-007718 rec'd 3/11/2014 – 47 animals, 235 samples (No Project given. Bison tissue/blood)

14-009113 rec'd 3/20/2014 – 6 animals, 15 samples (GonaCon, Bison blood/milk/swabs)

14-009804 rec'd 3/27/2014 – 11 animals, ~104 samples (VOC, Bison tissue/blood)

14-009805 rec'd 3/27/2014 – 10 animals, ~104 samples (VOC, bison tissue/blood)

14-015982, rec'd 5/20/2014 – 28 animals, 51 samples (GonaCon, Bison swabs/blood)

14-016015 rec'd 5/20/2014 – 22 animals, 78 samples (GonaCon, Bison cow/calf)

14-016090 rec'd 5/20/2014 -- 5 animals, ?5 samples (GonaCon, Bison blood)

14-016302 rec'd 5/22/2014 – 6 animals, 20 samples (Natural Infection Elk, tissues/blood)

14-017501 rec'd 6/3/2014 – 6 animals, 16 samples (GonaCon, Bison cow/calf)

14-018583 rec'd 6/12/2014 – 14 animals, 25 samples (Natural Infection Elk, blood and swabs)

14-018582 rec'd 6/12/2014 – 20 animals, 20 samples (No Project given. Bison degenerate embryos) =~673 samples (from WildIT) in the freezer atm.

Plus two accessions we should get tomorrow. One of which Matt indicated was priority... bull bison semen/blood. We will try to get those processed tomorrow afternoon or Friday after we set up 4 more pigs.

The order above is how the samples are logged in, and the order we have them set up to process. Ahead of you still...

We are about halfway through the 30 TN pigs (210 samples) that we got last week.

We were about 2/3 through the 40 sero-pos MT Bison (~160 samples) from John Treanor.

In between, we've been keeping up with other diagnostic program and user fee samples... which have priority over the research samples.

It is really hard to give an estimate on the turnaround for these... as it totally depends on what else comes in the door and how many positives we have (If a technician is doing identifications then they are not processing).

If you would like to prioritize the list above... we will certainly try to accommodate. It does take longer to do tissues than it does swabs, etc. Those samples we will go through fairly quickly once we get to them.

I do apologize for the delays! We are working though things as quickly as we can.

-Chris

From: Frey, Rebecca K - APHIS
Sent: Wednesday, June 18, 2014 3:22 PM
To: Quance, Christine R - APHIS
Subject: test results
Hi Chris!

We are looking for some culture results.....sorry, I know, you are likely awfully busy there.....just wondering if you had a sense of when we might get results from submissions in February and March? We are probably going to have about 2 more shipments for this year.....if we can get the bison to cooperate.

Thanks,

Becky

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: [Clarke, Patrick R. \(APHIS\)](#)
To: [Frey, Rebecca K \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: RE: Testing gonacon bufs
Date: Monday, August 29, 2011 2:45:01 PM

What are everyone's availability on Thursday Sept 1st for a conf call....talking about bull bison study, future of seropositive bulls, GonaCon study, etc.???? Becky and I have the day open...

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

-----Original Message-----

From: Frey, Rebecca K (APHIS)
Sent: Monday, August 29, 2011 2:06 PM
To: Clarke, Patrick R. (APHIS); McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Nol, Pauline (APHIS); (b) (6) @aol.com'
Cc: Herriott, Donald E (APHIS)
Subject: Testing gonacon bufs

Hi,

Just following up on a date to retest our GonaCon animals, which we originally intended to do end of Sept. Sept.21 or 22, or the following week? If anyone wants to join from Fort Collins we would be happy! We will likely retest then in early November, maybe preg check just to be sure, unless we have more positives, in which case we will need to test again in December. We plan to have positives and negatives separated before any new animals arrive in 2012.

From: [Clarke, Patrick R. \(APHIS\)](#)
To: [McCollum, Matthew P \(APHIS\)](#); [Frey, Rebecca K \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: RE: Testing gonacon bufs
Date: Wednesday, August 31, 2011 4:32:14 PM

11 it is tomorrow Thursday the 1st call in number: (b) (6) code; (b) (6)

P. Ryan Clarke, DVM
Regional Epidemiologist-GYA
USDA/APHIS/VS/WR
Belgrade, Montana
406-388-5162

-----Original Message-----

From: McCollum, Matthew P (APHIS)
Sent: Wednesday, August 31, 2011 11:06 AM
To: Clarke, Patrick R. (APHIS); Frey, Rebecca K (APHIS); Rhyan, Jack C (APHIS); Nol, Pauline (APHIS)
Subject: RE: Testing gonacon bufs

How about 11ish on Thursday?

-----Original Message-----

From: Clarke, Patrick R. (APHIS)
Sent: Monday, August 29, 2011 2:45 PM
To: Frey, Rebecca K (APHIS); McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Nol, Pauline (APHIS)
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Sent: Monday, August 29, 2011 2:06 PM
To: Clarke, Patrick R. (APHIS); McCollum, Matthew P (APHIS); Rhyan, Jack C (APHIS); Nol, Pauline (APHIS); (b) (6) @aol.com'
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From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: RE: update of GonaCon Study IACUC
Date: Wednesday, December 11, 2013 3:22:00 PM
Attachments: [Study Protocol Renewal Bison Gonacon Montana.docx](#)

Hey Becky,

Could you fill in the gaps (comments) on this renewal form? Then I'll have Jack sign it and send it on to Ryan.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Tuesday, December 10, 2013 9:42 AM
To: Nol, Pauline - APHIS
Cc: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS
Subject: update of GonaCon Study IACUC

Hi Pauline!

It is only about -30 wind chill here, 40 -70 mph gusts, AWESOME! Can't wait for you all to get here!

I am updating the permits, and see that we have an expiration on our IACUC of May 2014. I am sure we will need to update signatures for another 3 yr. period in order to keep our permits active. Can you initiate a new form for signatures on that? I only have the PDF copy.

Thanks!

Becky

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaConTM, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

2. Action needed:

 Project Completed

 Project Never Initiated

 X Project On-going/Active: renew as approved

 Project On-going/Active: renew with minor revisions

 Project Not Yet Initiated or is Inactive: renewal requested

Anticipated start date

3. Protocol Changes

In the upcoming year will you implement any changes to the animal component of the project that differ from those in the original (or subsequent) approval by the IACUC (e.g. changes to animal procedures, number of animals needed, or project objectives)?

Yes No X

3. Animal Use and Procedure alternatives since the last IACUC approval

a. Have alternatives to the use of animals become available that could be substituted to achieve specific project aims? Yes No X

b. Have alternatives that are potentially less painful or distressful to animals become available that could be used to achieve specific project aims? Yes No X

If you answered yes to either question, please provide a description below or attach one.

N/A

Animal usage (please complete the following box):

Commented [pn1]: Becky, can you fill out the first column?

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	104			
2. Number of animals used during first IACUC approval year				
3. Number of animals used during second IACUC approval year (enter 0 if in future)				
4. Number of animals used during third approval year				

Note: Additional animals will be collected (up to) in winter/spring 2014 to replicate the study as described in the original protocol.

Commented [pn2]: Based on 104 minus how many we started with in 2011, what would this number be?

Study Director _____ Date _____

Concur

IACUC Chair _____ Date _____

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: RE: update of GonaCon Study IACUC
Date: Wednesday, December 11, 2013 2:24:00 PM

Hey Becky,

I'll only get the ACUC renewed on condition that it's over 20 degrees when we travel up there.

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

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Becky

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: Re: update of GonaCon Study IACUC
Date: Thursday, December 12, 2013 7:33:16 AM

It will probably be over 20 degrees, but I won't guarantee there won't be 70 mph winds! So glad you didn't mention that !! :-)

Becky
USDA APHIS VS
Sent from my iPhone

On Dec 11, 2013, at 2:24 PM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Hey Becky,
I'll only get the ACUC renewed on condition that it's over 20 degrees when we travel up there.
Pauline
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
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Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
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From: Frey, Rebecca K - APHIS
Sent: Tuesday, December 10, 2013 9:42 AM
To: Nol, Pauline - APHIS
Cc: Rhyen, Jack C - APHIS; Clarke, Patrick R. - APHIS
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Becky
Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: update of GonaCon Study IACUC
Date: Thursday, December 12, 2013 3:46:11 PM
Attachments: [Study Protocol Renewal Bison Gonacon Montana-rkf.docx](#)

Hi,

So I assume that the animals used in years 1, 2 and 3 are not accumulated to 104....they are the same 40 head continuing in the study. We have given YNP 59 for a number needed in order to have plenty of negative bulls for the future. We actually collected 49 the first year, 8 are left not being used, and we hope to not need them since they are "aged". I did not include them on the count.

Becky

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Wednesday, December 11, 2013 3:23 PM
To: Frey, Rebecca K - APHIS
Subject: RE: update of GonaCon Study IACUC

Hey Becky,

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Thanks!

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Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
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From: Frey, Rebecca K - APHIS
Sent: Tuesday, December 10, 2013 9:42 AM
To: Nol, Pauline - APHIS
Cc: Rhyen, Jack C - APHIS; Clarke, Patrick R. - APHIS
Subject: update of GonaCon Study IACUC

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Becky

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

2. Action needed:

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Yes No X

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a. Have alternatives to the use of animals become available that could be substituted to achieve specific project aims? Yes No X

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If you answered yes to either question, please provide a description below or attach one.

N/A

Animal usage (please complete the following box):

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Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	104			
2. Number of animals used during first IACUC approval year	40			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	40			
4. Number of animals used during third approval year	42			

Note: Additional animals will be collected (up to 62) in winter/spring 2014 to replicate the study as described in the original protocol.

Commented [pn2]: Based on 104 minus how many we started with in 2011, what would this number be?

Study Director _____ Date _____

Concur

IACUC Chair _____ Date _____

From: [Rhyan, Jack C - APHIS](#)
To: [Ahola, Sara C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: update on activities?
Date: Monday, November 14, 2016 4:33:40 PM
Attachments: [Research updateConfCall8-4-16.pptx](#)

Sarah, here is one I used recently. Hope you can glean from it.
Jack

From: Ahola, Sara C - APHIS
Sent: Monday, November 14, 2016 4:08 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: update on activities?

Hello Jack and Pauline,

I have to give a presentation on Wednesday to Foreign Animal Disease Diagnosticians in the Colorado area. Would you be able to provide me with one or two slides on your current activities – GonaCon trial, etc. I'll give you credit of course, and don't worry about formatting them – I'll incorporate. This is only if you have time. . . . maybe whatever Pauline used in Pocatello?

~Sara

Sara C. Ahola, DVM, MA-Econ.
Veterinary Medical Officer-Epidemiology
United States Department of Agriculture
APHIS-Veterinary Services: Surveillance, Preparedness & Response Services
Cattle Health Staff
2150 Centre Ave
Building B, Mail stop 3E13
Fort Collins, CO 80526
Office: 970-494-7441
Cell: (b) (6)
Sara.C.Ahola@aphis.usda.gov



United States Department of Agriculture

Veterinary Services

Tools to detect and eliminate
brucellosis from wild populations:
current research

Jack Rhyan
Wildlife/Livestock Disease
Investigations Team

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
October 25, 2015



Wildlife/livestock Disease Investigations Team



Pauline Nol



Karl Held



Matt McCollum

Samantha Bruce

- “Developing science-based solutions to disease problems at the wildlife/domestic animal interface”

Tools

- Disease detection: Volatile Organic Compound (VOC) analysis
- Disease elimination:
 - Immun contraceptive vaccine (GonaCon™)
 - Brucella vaccines
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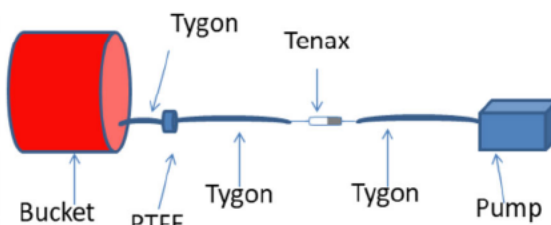
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2.2 L/min for 2 min



Technion-Israel Institute
of Technology-Haick
Laboratory

Methods: Breath Analysis

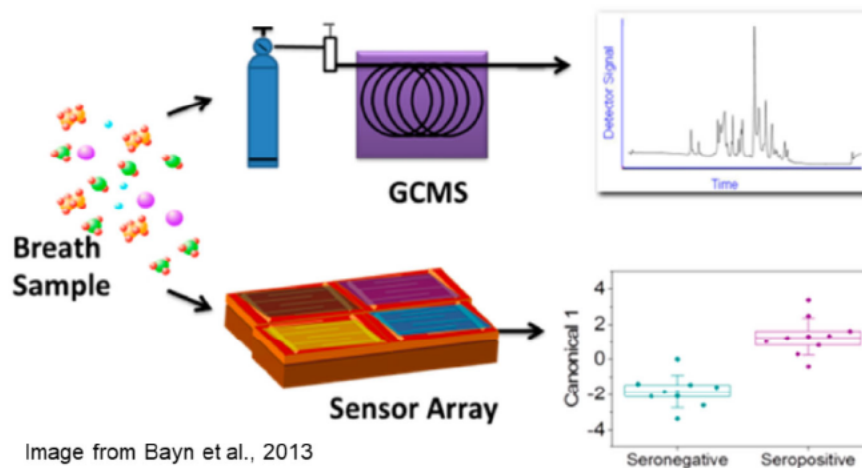
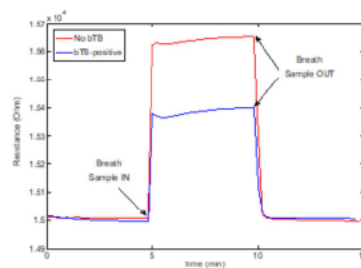
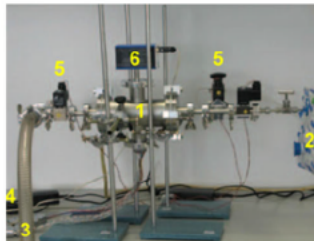


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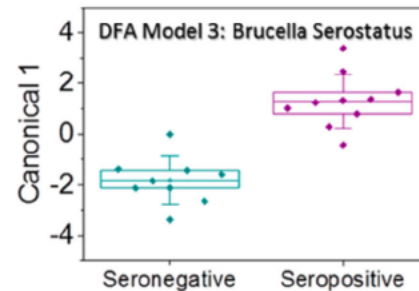
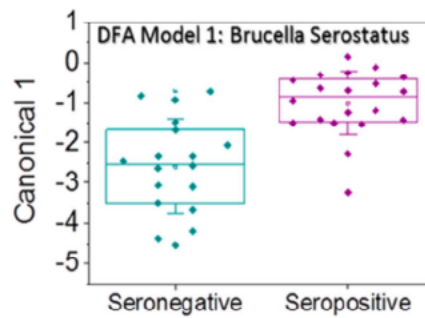
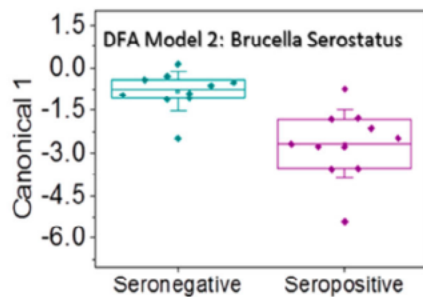
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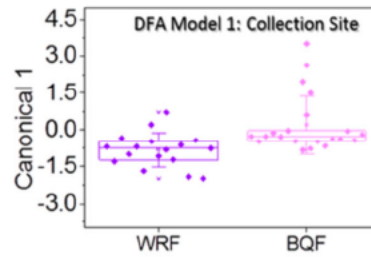
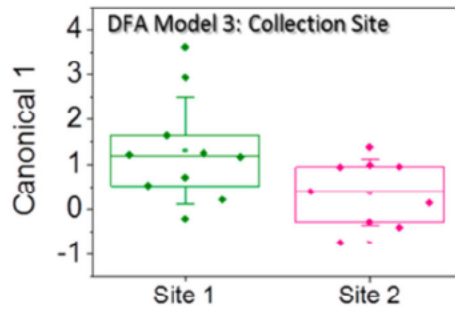
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United States Department of Agriculture

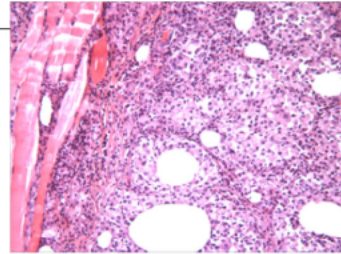
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United States Department of Agriculture

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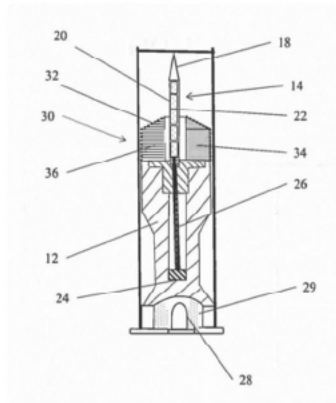
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25

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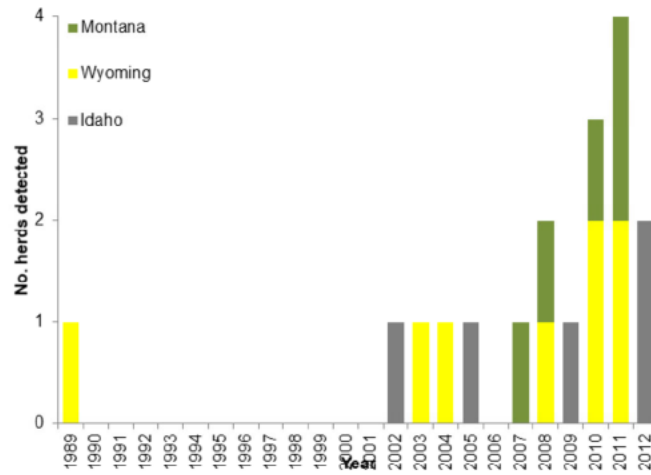


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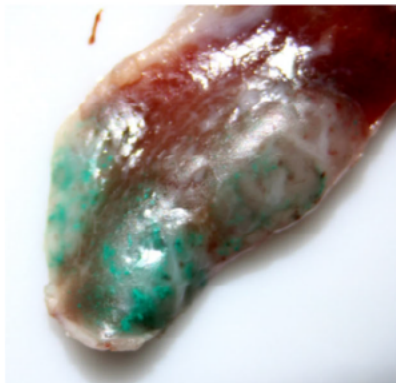
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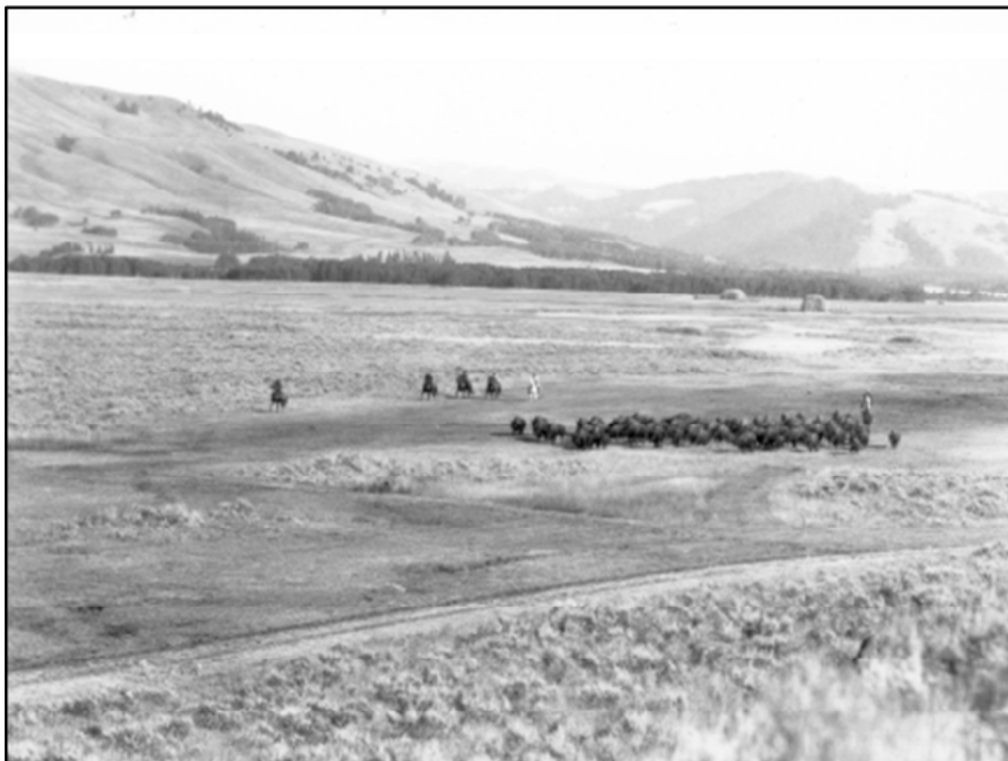
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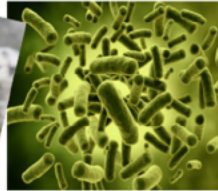
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Questions?



From: [Rhyan, Jack C - APHIS](#)
To: [Ahola, Sara C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: update on activities?
Date: Monday, November 14, 2016 4:33:40 PM
Attachments: [Research updateConfCall8-4-16.pptx](#)

Sarah, here is one I used recently. Hope you can glean from it.
Jack

From: Ahola, Sara C - APHIS
Sent: Monday, November 14, 2016 4:08 PM
To: Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
Subject: update on activities?

Hello Jack and Pauline,

I have to give a presentation on Wednesday to Foreign Animal Disease Diagnosticians in the Colorado area. Would you be able to provide me with one or two slides on your current activities – GonaCon trial, etc. I'll give you credit of course, and don't worry about formatting them – I'll incorporate. This is only if you have time. . . . maybe whatever Pauline used in Pocatello?

~Sara

Sara C. Ahola, DVM, MA-Econ.
Veterinary Medical Officer-Epidemiology
United States Department of Agriculture
APHIS-Veterinary Services: Surveillance, Preparedness & Response Services
Cattle Health Staff
2150 Centre Ave
Building B, Mail stop 3E13
Fort Collins, CO 80526
Office: 970-494-7441
Cell: (b) (6)
Sara.C.Ahola@aphis.usda.gov



United States Department of Agriculture

Veterinary Services

Tools to detect and eliminate
brucellosis from wild populations:
current research

Jack Rhyan
Wildlife/Livestock Disease
Investigations Team

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
October 25, 2015



Wildlife/livestock Disease Investigations Team



Pauline Nol



Karl Held



Matt McCollum

Samantha Bruce

- “Developing science-based solutions to disease problems at the wildlife/domestic animal interface”

Tools

- Disease detection: Volatile Organic Compound (VOC) analysis
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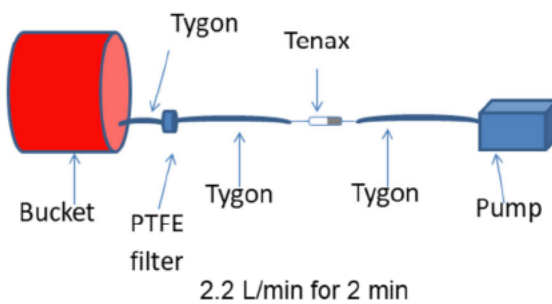
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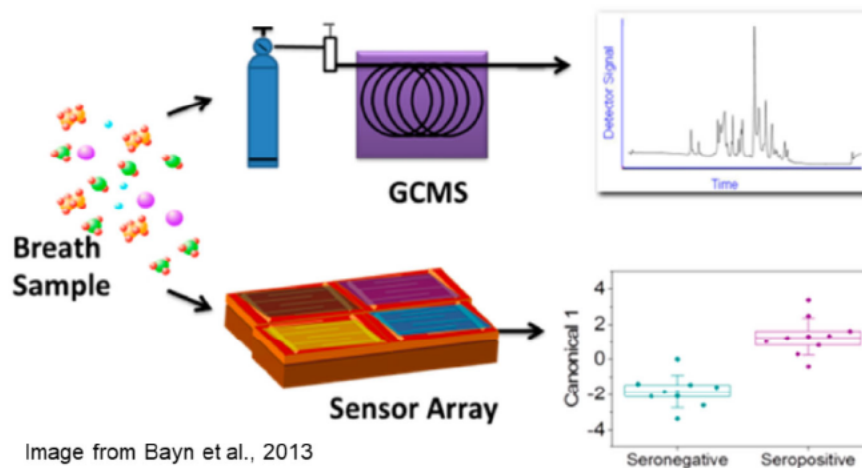
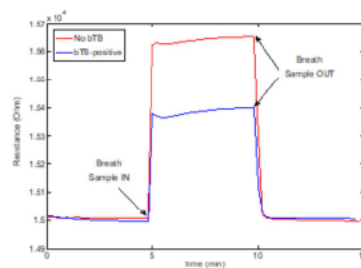
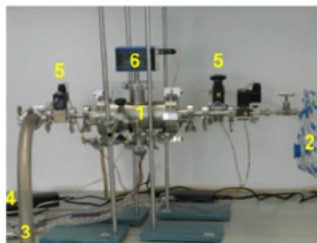


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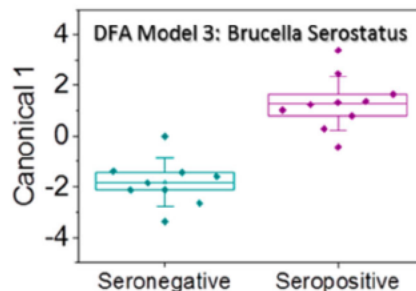
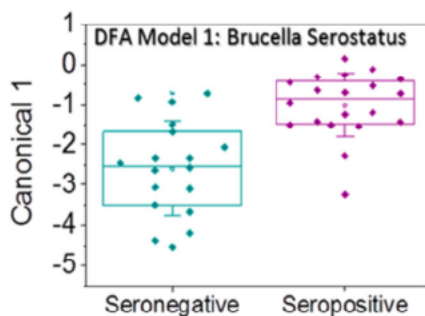
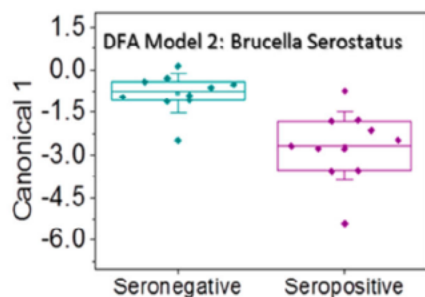
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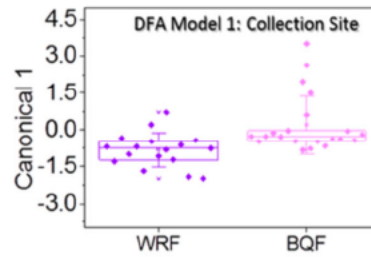
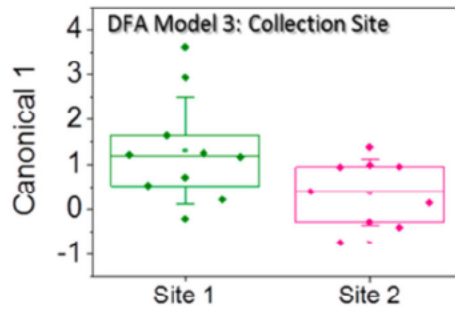
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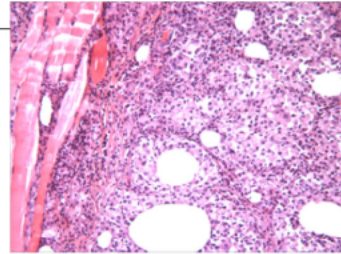
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United States Department of Agriculture

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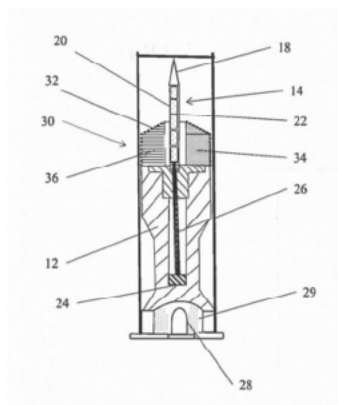
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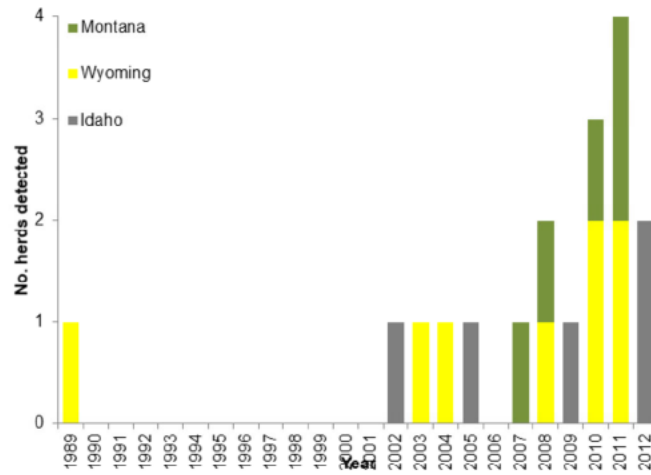


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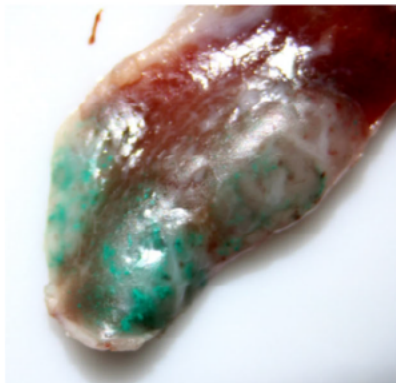
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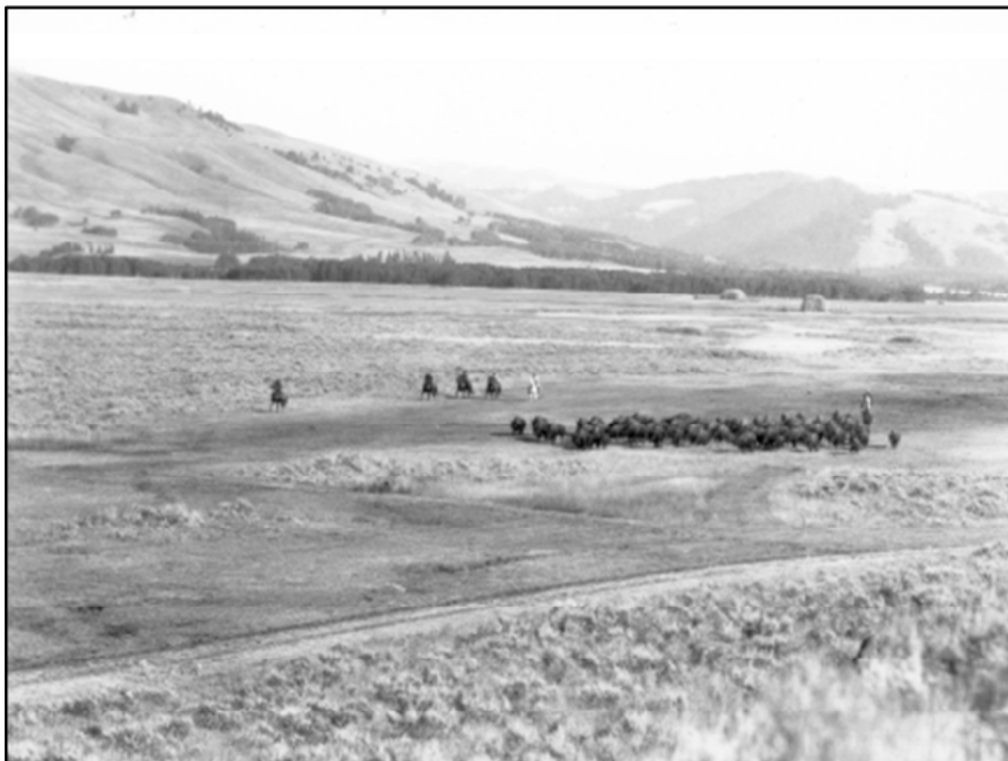
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From: Quance, Christine R - APHIS
To: Nol, Pauline - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
Date: Monday, January 28, 2013 3:55:48 PM

Thanks!

So, for this study...how often are you anticipating sampling for culture and how many animals each time?

From: Nol, Pauline - APHIS
Sent: Thursday, January 24, 2013 1:47 PM
To: Quance, Christine R - APHIS; Hennager, Steven G - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
Hi Chris,

All the bison are on an immunocontraceptive study using GonaCon, which is a GnRH-based vaccine. We are trying to determine whether contracepting brucella+ bison reduces bacterial shedding.

Thanks!

Pauline

From: Quance, Christine R - APHIS
Sent: Thursday, January 24, 2013 11:24 AM
To: Nol, Pauline - APHIS; Hennager, Steven G - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
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We did already get these! (Only discrepancy was two vag swabs were rec'd for R15 instead of 1)

What is the GnRH study??

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Thanks!

Pauline

From: UPS Quantum View [<mailto:auto-notify@ups.com>]
Sent: Thursday, January 24, 2013 8:43 AM
To: Nol, Pauline - APHIS
Subject: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366

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Important Delivery Information

Tracking Number: [1Z1Y773V1597261366](#)

Delivery Date / Time: 24-January-2013 / 9:06 AM

Delivery Location Left At: DOCK
Signed by: BECK

Shipment Detail

Ship To:

Christine Quance
USDA / APHIS / VS/NVSL
1920 DAYTON AVE
AMES
IA
50010
US

Number of Packages: 1**UPS Service:** NEXT DAY AIR EARLY AM**Hazardous Materials:** YES**Weight:** 9.0 LBS

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From: [Nol, Pauline - APHIS](#)
To: [Quance, Christine R - APHIS](#); [Hennager, Steven G - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
Date: Thursday, January 24, 2013 12:46:00 PM

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All the bison are on an immunocontraceptive study using GonaCon, which is a GnRH-based vaccine. We are trying to determine whether contracepting brucella+ bison reduces bacterial shedding.

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Hazardous Materials: YES

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[Contact UPS](#)



From: [Nol, Pauline - APHIS](#)
To: [Quance, Christine R - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#)
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
Date: Monday, January 28, 2013 4:30:00 PM

Hi Chris,

We plan to whole herd (39 animals) test once per year (blood and vag. Swabs) in Jan/Feb. However, during calving season, we will be collecting vaginal swabs, blood, and milk from cows, and blood and conjunctival swabs from all calves. If placenta and tissues from aborted fetuses are available, they will be submitted for culture as well. This year there are 22 pregnant cows that will either calve or abort. We may be submitting some samples from our 2 bulls as well once per year.

Let me know if you have any more questions.

Thanks!

Pauline

From: Quance, Christine R - APHIS
Sent: Monday, January 28, 2013 3:56 PM
To: Nol, Pauline - APHIS
Cc: Rhyan, Jack C - APHIS
Subject: RE: UPS Delivery Notification, Tracking Number 1Z1Y773V1597261366
Thanks!

So, for this study...how often are you anticipating sampling for culture and how many animals each time?

From: Nol, Pauline - APHIS
Sent: Thursday, January 24, 2013 1:47 PM
To: Quance, Christine R - APHIS; Hennager, Steven G - APHIS
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Signed by: BECK

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Christine Quance
USDA / APHIS / VS/NVSL
1920 DAYTON AVE
AMES
IA
50010
US

Number of Packages: 1

UPS Service: NEXT DAY AIR EARLY AM

Hazardous Materials: YES

Weight: 9.0 LBS

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From: [Quance, Christine R - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Friday, June 12, 2015 12:15:59 PM

Yes you did. Thanks for checking.

And yeah on the blood tubes. We appreciate you guys bagging each sample though, made it much easier to contain.

You could try collecting blood in the SPS tubes (light yellow cap). Our method has always said heparin tubes, but I'm not sure who/when that was validated. Still have the same breakage potential with the SPS tubes, but they are meant for culture and might be worth a try or side-by-side.

As an FYI, I am looking at different blood culture systems. Not sure if we'll get one though, they are fairly pricey.

Chris

From: Nol, Pauline - APHIS
Sent: Friday, June 12, 2015 12:53 PM
To: Quance, Christine R - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Chris,

Did I address all the issues? I was on the road and not sure if I answered all of your questions.

I'm so bummed about the blood tubes! I guess since they never test positive anymore, it doesn't matter!:

(

Sorry this was such a mess!

Pauline

From: Quance, Christine R - APHIS
Sent: Wednesday, June 10, 2015 2:46 PM
To: Nol, Pauline - APHIS
Cc: Bartlett, Justin H - APHIS; Robbe Austerman, Suelee - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Ok. A few more questions and notifications.

The cow/calf (#50, AI3) samples- Did you want the calf feces tested for Johnes or only the cow? The submission form is not clear, but only the cow had a separate fecal sample for Johnes, so I assume it's only the cow, but please confirm.

We have two samples that are labeled differently than the submission form, please confirm which is correct:

5R18 – sample labeled 3R18

157 – sample labeled 57

We have at least 7 blood samples with broken tubes- these will NOT be processed:

420

69R

3R21
130
3R30
3R7
3R24

Thanks,
Chris

From: Nol, Pauline - APHIS
Sent: Wednesday, June 10, 2015 3:35 PM
To: Quance, Christine R - APHIS
Cc: Bartlett, Justin H - APHIS; Robbe Austerman, Suelee - APHIS
Subject: Re: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Yes they are gonacon animals. Thanks!!

Sent from my iPhone

On Jun 10, 2015, at 13:35, Quance, Christine R - APHIS <Christine.R.Quance@aphis.usda.gov> wrote:

Justin/Pauline,
Are these GonaCon animals? We need to indicate which project they are associated with.
Thanks!
Chris

From: Bartlett, Justin H - APHIS
Sent: Tuesday, June 09, 2015 3:25 PM
To: Nol, Pauline - APHIS; Robbe Austerman, Suelee - APHIS; Quance, Christine R - APHIS
Subject: FW: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Pauline here are the two boxes of samples you wanted shipped. The third box is the samples from the calf that was born on Friday and the cow.

Thanks,

Justin Bartlett
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6318
Fax: 970-266-6157

From: UPS Quantum View [<mailto:auto-notify@ups.com>]
Sent: Tuesday, June 09, 2015 2:00 PM
To: Bartlett, Justin H - APHIS
Subject: UPS Ship Notification, Tracking Number 1Z5368770194411368

<image001.jpg>

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Scheduled Delivery: 10-June-2015

Shipment Detail

Ship To:

USDA, APHIS, VS, NVSL
1920 Dayton Avenue
AMES
IA
500109602
US

Number of Packages: 3

UPS Service: NEXT DAY AIR

Hazardous Materials: YES

Weight: 22.0 LBS

Tracking Number: [1Z5368770194411368](http://www.ups.com/WebTracking/track?loc=en_US)

Reference Number 1: REF 16

Reference Number 2: #1

[Click here](http://www.ups.com/WebTracking/track?loc=en_US) to track if UPS has received your shipment or visit http://www.ups.com/WebTracking/track?loc=en_US on the Internet.

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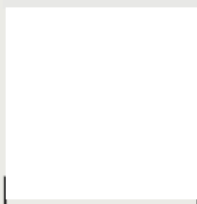
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From: [Quance, Christine R - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Bartlett, Justin H - APHIS](#); [Robbe Austerman, Suelee - APHIS](#)
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Wednesday, June 10, 2015 3:00:58 PM

Ok. Is the other one 57 or 157? Tubes and bag were labeled 57, paperwork has 157.

Two tubes were snapped totally in half. Others were crushed in one or more spots.

From: Nol, Pauline - APHIS
Sent: Wednesday, June 10, 2015 3:56 PM
To: Quance, Christine R - APHIS
Cc: Bartlett, Justin H - APHIS; Robbe Austerman, Suelee - APHIS
Subject: Re: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Hi Chris,
Johne's just for the cow.
3R18 is be correct. Sorry!
Bad on the blood tubes!!! Were they cracked or crushed??
Pauline

Sent from my iPhone

On Jun 10, 2015, at 14:45, Quance, Christine R - APHIS <Christine.R.Quance@aphis.usda.gov> wrote:

Ok. A few more questions and notifications.

The cow/calf (#50, AI3) samples- Did you want the calf feces tested for Johnes or only the cow?
The submission form is not clear, but only the cow had a separate fecal sample for Johnes, so I assume it's only the cow, but please confirm.

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420
69R
3R21
130
3R30
3R7
3R24

Thanks,
Chris

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Yes they are gonacon animals. Thanks!!

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Justin/Pauline,

Are these GonaCon animals? We need to indicate which project they are associated with.

Thanks!

Chris

From: Bartlett, Justin H - APHIS

Sent: Tuesday, June 09, 2015 3:25 PM

To: Nol, Pauline - APHIS; Robbe Austerman, Suelee - APHIS; Quance, Christine R - APHIS

Subject: FW: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Pauline here are the two boxes of samples you wanted shipped. The third box is the samples from the calf that was born on Friday and the cow.

Thanks,

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USDA-APHIS-VS-STAS

National Wildlife Research Center

4101 LaPorte Ave.

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Office: 970-266-6318

Fax: 970-266-6157

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Sent: Tuesday, June 09, 2015 2:00 PM

To: Bartlett, Justin H - APHIS

Subject: UPS Ship Notification, Tracking Number 1Z5368770194411368



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USDA, APHIS, VS, NVSL

1920 Dayton Avenue
AMES
IA
500109602
US

Number of Packages: 3

UPS Service: NEXT DAY AIR

Hazardous Materials: YES

Weight: 22.0 LBS

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Reference Number 1: REF 16

Reference Number 2: #1

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[<image002.jpg>](#)

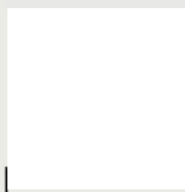
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Cc: [Bartlett, Justin H - APHIS](#); [Robbe Austerman, Suelee - APHIS](#)
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Wednesday, June 10, 2015 2:45:47 PM

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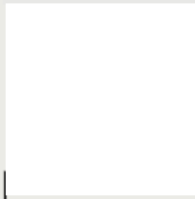
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To: [Bartlett, Justin H - APHIS](#); [Nol, Pauline - APHIS](#); [Robbe Austerman, Suelee - APHIS](#)
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Wednesday, June 10, 2015 1:35:10 PM

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Number of Packages: 3**UPS Service:** NEXT DAY AIR**Hazardous Materials:** YES**Weight:** 22.0 LBS**Tracking Number:** [1Z5368770194411368](http://www.ups.com/WebTracking/track?loc=en_US)**Reference Number 1:** REF 16**Reference Number 2:** #1

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From: [Quance, Christine R - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Friday, June 12, 2015 12:32:07 PM

Hmm.. I wonder if they clot otherwise. If they are frozen soon after collection then we shouldn't have that issue.

The WHO media might be ok too, but it would be diluting the sample. However that is better than no sample at all.

From: Nol, Pauline - APHIS
Sent: Friday, June 12, 2015 1:27 PM
To: Quance, Christine R - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

I should have transferred to cryotubes, but I guess I was thinking we had discussed that and sending them directly in the heparin collection tubes was okay.

That's an interesting idea to try the SPS tubes. But BD says you have to transfer the blood to a bottle within 4 hours. Don't know why. We can still transfer to cryotubes.

What if we transfer the blood to a tube with a ml of WHO media? Have we tried that?

From: Quance, Christine R - APHIS
Sent: Friday, June 12, 2015 12:16 PM
To: Nol, Pauline - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Yes you did. Thanks for checking.

And yeah on the blood tubes. We appreciate you guys bagging each sample though, made it much easier to contain.

You could try collecting blood in the SPS tubes (light yellow cap). Our method has always said heparin tubes, but I'm not sure who/when that was validated. Still have the same breakage potential with the SPS tubes, but they are meant for culture and might be worth a try or side-by-side.

As an FYI, I am looking at different blood culture systems. Not sure if we'll get one though, they are fairly pricey.

Chris

From: Nol, Pauline - APHIS
Sent: Friday, June 12, 2015 12:53 PM
To: Quance, Christine R - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Chris,

Did I address all the issues? I was on the road and not sure if I answered all of your questions.

I'm so bummed about the blood tubes! I guess since they never test positive anymore, it doesn't matter!:
(

Sorry this was such a mess!
Pauline

From: Quance, Christine R - APHIS
Sent: Wednesday, June 10, 2015 2:46 PM
To: Nol, Pauline - APHIS
Cc: Bartlett, Justin H - APHIS; Robbe Austerman, Suelee - APHIS
Subject: RE: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Ok. A few more questions and notifications.

The cow/calf (#50, AI3) samples- Did you want the calf feces tested for Johnes or only the cow? The submission form is not clear, but only the cow had a separate fecal sample for Johnes, so I assume it's only the cow, but please confirm.

We have two samples that are labeled differently than the submission form, please confirm which is correct:

5R18 – sample labeled 3R18

157 – sample labeled 57

We have at least 7 blood samples with broken tubes- these will NOT be processed:

420

69R

3R21

130

3R30

3R7

3R24

Thanks,
Chris

From: Nol, Pauline - APHIS
Sent: Wednesday, June 10, 2015 3:35 PM
To: Quance, Christine R - APHIS
Cc: Bartlett, Justin H - APHIS; Robbe Austerman, Suelee - APHIS
Subject: Re: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Yes they are gonacon animals. Thanks!!

Sent from my iPhone

On Jun 10, 2015, at 13:35, Quance, Christine R - APHIS <Christine.R.Quance@aphis.usda.gov> wrote:

Justin/Pauline,

Are these GonaCon animals? We need to indicate which project they are associated with.

Thanks!

Chris

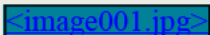
From: Bartlett, Justin H - APHIS
Sent: Tuesday, June 09, 2015 3:25 PM
To: Nol, Pauline - APHIS; Robbe Austerman, Suelee - APHIS; Quance, Christine R - APHIS
Subject: FW: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples

Pauline here are the two boxes of samples you wanted shipped. The third box is the samples from the calf that was born on Friday and the cow.

Thanks,

Justin Bartlett
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6318
Fax: 970-266-6157

From: UPS Quantum View [<mailto:auto-notify@ups.com>]
Sent: Tuesday, June 09, 2015 2:00 PM
To: Bartlett, Justin H - APHIS
Subject: UPS Ship Notification, Tracking Number 1Z5368770194411368



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Ship To:
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1920 Dayton Avenue
AMES
IA
500109602
US

Number of Packages: 3
UPS Service: NEXT DAY AIR
Hazardous Materials: YES
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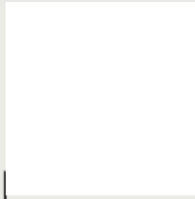
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From: [Nol, Pauline - APHIS](#)
To: [Quance, Christine R - APHIS](#)
Cc: [Bartlett, Justin H - APHIS](#); [Robbe Austerman, Suelee - APHIS](#)
Subject: Re: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Date: Wednesday, June 10, 2015 2:35:23 PM

Yes they are gonacon animals. Thanks!!

Sent from my iPhone

On Jun 10, 2015, at 13:35, Quance, Christine R - APHIS <Christine.R.Quance@aphis.usda.gov> wrote:

Justin/Pauline,
Are these GonaCon animals? We need to indicate which project they are associated with.
Thanks!
Chris

From: Bartlett, Justin H - APHIS
Sent: Tuesday, June 09, 2015 3:25 PM
To: Nol, Pauline - APHIS; Robbe Austerman, Suelee - APHIS; Quance, Christine R - APHIS
Subject: FW: UPS Ship Notification, Tracking Number 1Z5368770194411368 for samples
Pauline here are the two boxes of samples you wanted shipped. The third box is the samples from the calf that was born on Friday and the cow.
Thanks,
Justin Bartlett
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6318
Fax: 970-266-6157

From: UPS Quantum View [<mailto:auto-notify@ups.com>]
Sent: Tuesday, June 09, 2015 2:00 PM
To: Bartlett, Justin H - APHIS
Subject: UPS Ship Notification, Tracking Number 1Z5368770194411368

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From: [Clark, Larry - APHIS](#)
To: [Tobin, Mark E - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: RE: VS project update
Date: Friday, January 18, 2013 11:43:47 AM

Jack & Pauline.
Thank you very much!.
Larry

Larry Clark, Ph.D.
Director
National Wildlife Research Center
USDA-APHIS-WS
4101 La Porte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6036
Fx: (970) 266-6040
Mobile: (b) (6)
larry.clark@aphis.usda.gov
http://www.aphis.usda.gov/wildlife_damage/nwrc/

From: Tobin, Mark E - APHIS
Sent: Friday, January 18, 2013 11:00 AM
To: Clark, Larry - APHIS
Subject: FW: VS project update
Larry,

FYI, Jack Rhyan and Pauline Nol have brought all their studies up to date with the NWRC QA unit.
met

Mark E. Tobin, Ph.D.
Assistant Director
National Wildlife Research Center
USDA-APHIS-WS
4101 La Porte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6035
Fx: (970) 266-6040
Mobile: (b) (6)
http://www.aphis.usda.gov/wildlife_damage/nwrc/

From: Greiner, Laura B - APHIS
Sent: Friday, January 18, 2013 10:57 AM
To: Tobin, Mark E - APHIS
Subject: VS project update
Mark

An update on Jack/Pauline is listed below. Contact me if you have any questions.

Laura Greiner

<i>Study Director</i>	<i>#</i>	<i>Type</i>	<i>Title</i>	<i>Experiment Start Date</i>	<i>Experiment Term Date</i>	<i>Archive Due Date</i>	<i>Status</i>
Nol	1316	NON	Investigation of a recombinant Brucella abortus strain RB51 in elk	09/01/05	01/30/06	06/01/13	on-going
	1996	NON	Efficacy of B. suis VRTS1 / contraceptive vaccine in swine				<i>not initiated</i>
Rhyan	1858	NON	Evaluation of GonaCon, an immunocontraceptive vaccine, as a means of decreasing shedding of Brucella abortus in bison	04/15/12	10/01/17	10/01/19	on-going
	1923	NON	Evaluation of GonaCon, an immunocontraceptive vaccine, in free-ranging bison: A pilot study	11/07/11	11/01/17	11/01/19	on-going

Note (original list is below):

QA-1316 Nol amended the study completion date to June 2013 to allow time for archiving.

QA-1463 Nol cancelled with amendment 3 – she inherited this study and we were unable to locate any data.

QA-823 Rhyan archived 1/17/13.

<i>Study Director</i>	<i>#</i>	<i>Type</i>	<i>Title</i>	<i>Experiment Start Date</i>	<i>Experiment Term Date</i>	<i>Archive Due Date</i>	<i>Status</i>
Nol	1316	NON	Investigation of a recombinant Brucella abortus strain RB51 in elk	09/01/05	01/30/06	01/01/09	overdue
	1463	NON	Second generation CWD vaccine development and PrPres specific monoclonal antibody production	04/30/07	12/30/08	10/30/09	overdue
	1996	NON	Efficacy of B. suis VRTS1 / contraceptive vaccine in swine				<i>not initiated</i>
Rhyan	823	NON	Development of oral RB51 for use in free-ranging animals: Phase I Mouse model	07/30/00	12/30/00	12/30/02	overdue
	1858	NON	Evaluation of GonaCon, an immunocontraceptive vaccine, as a means of decreasing shedding of Brucella abortus in bison	04/15/12	10/01/17	10/01/19	on-going
	1923	NON	Evaluation of GonaCon, an immunocontraceptive vaccine, in free-ranging bison: A pilot study	11/07/11	11/01/17	11/01/19	on-going

From: [McCollum, Matthew P - APHIS](#)
To: [Hastings, Bruce](#)
Cc: david_c_lucas@fws.gov; [Tom Ronning](#); [Rhyen, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: RE: Well met
Date: Wednesday, April 30, 2014 11:27:18 AM
Attachments: [Contraception of BisonJWD.PDF](#)
[ContraceptionArtJZVM2013.pdf](#)

Good Morning,

Bruce and I briefly discussed bison and the issues associated with population growth. As I understand it, those issues are exacerbated by the status of the RMA as a superfund site and the present disposition of any animals that are removed from that area dictate that they may not be used for human consumption. I recognize that you are not interested in long term sterilization of the animals and that you are managing them as a meta population and selecting for certain genetics. We could potentially be of assistance to each other by setting up a GnRH trial using your animals. We are looking to get GnRH approved by the FDA for use in bison. It is already approved for use in deer. Until we get approval, those cows that receive the experimental drug cannot be used for human consumption per FDA rules. We have completed three small studies using GnRH in bison and have two ongoing studies at present. Attached are two publications detailing our previous work.

Ongoing studies:

Sand Dunes Nature Conservancy GnRH trial: We dosed 10 cows with the medium dose of GnRH. The first year was moderately successful with 6/10 being open versus 1/9 controls. The second year was better with 9/10 contracepted vs 3/9 open controls. We are presently in the 3rd year of the study.

GnRH as a brucellosis treatment to prevent transmission: The goal of this study is to evaluate GnRH vaccine as a treatment of infected animals to prevent disease transmission. If infected bison do not get pregnant, they should have a lower potential for shedding and transmitting brucellosis. So far we have started the study and preliminary results are promising.

We recognize the difficulty of the situation you are in, but perhaps there is a potential to slow the recruitment rate of your animals and also get some good science done. We'd love to have a larger vaccinate group that is also matched with a control group. I don't know the numbers or age classes of animals you have, but one option would be to use your genetic information and then pick a vaccinate group that is well represented genetically and a control group with more valuable genetics.

This may or may not be a good fit. Maybe it'd be best to keep the pressure on to push the inertia of the regulatory agencies to make a decision and remove the restrictions from your animals. Maybe it'd be best for you to expand the range where you have your animals and have more. On the other hand, it might work well to slow the process down a bit. The GnRH vaccine is not perfected yet in bison. We need more replicates to better assess the vaccine. The use of the vaccine is for a limited audience- most people would want to grow and grow their herd and be able to sell them for profit, but there are some groups that have conservation or, perhaps more accurately, display herds that would be easier to manage if they had a tool to slow down recruitment.

At this point, all we want to do is start a dialog and put the information out there for you to make an informed decision.

We look forward to hearing from you,

Matt McCollum

Wildlife Disease Biologist
USDA/APHIS/VS

Wildlife/Livestock Disease Investigations Team
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 Office

(b) (6) Mobile

From: Hastings, Bruce [mailto:bruce_hastings@fws.gov]
Sent: Tuesday, April 29, 2014 10:10 AM
To: Rhyan, Jack C - APHIS
Cc: McCollum, Matthew P - APHIS; david_c_lucas@fws.gov; Tom Ronning
Subject: Re: Well met

Jack,

I asked Matt to send me some information on this technique, which he will do tomorrow when he returns from South Dakota. However, we are trying to resolve our potential population problems by (1) opening more rangeland for grazing and (2) demonstrating to the regulators that the bison are not contaminated and therefore restrictions on their consumption can be lifted. Hopefully, we will not need to use contraception, but we always need to know our options. I look forward to reading what Matt sends, but we are not ready to initiate birth control at this time.

Bruce

On Tue, Apr 29, 2014 at 9:44 AM, Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov> wrote:

Bruce,

Matt told me of your conversation yesterday about bison contraception. We would like to visit with you more about potential collaboration.

Please give me a call if you get a chance.

Thanks.

Jack 970 266-6140

From: McCollum, Matthew P - APHIS
Sent: Tuesday, April 29, 2014 7:39 AM
To: bruce_hastings@fws.gov

Cc: Rhyan, Jack C - APHIS

Subject: Well met

Dear Bruce,

It was nice to meet you yesterday and chat a bit about bison. I need to run up to South Dakota today to pick up a squeeze chute, so I'll follow up more tomorrow in regards to our conversation about GnRH.

Best,

Matt McCollum

Wildlife Disease Biologist
USDA/APHIS/VS
Wildlife/Livestock Disease Investigations Team
4101 Laporte Ave
Fort Collins, CO 80521
(970)266-6233 Office
(b) (6) Mobile

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Bruce Hastings
Deputy Project Leader
Rocky Mountain Arsenal NWR Complex
6550 Gateway Road, Building 129
Commerce City, CO 80022-1748
303-289-0533

CONTRACEPTION OF BISON BY GnRH VACCINE: A POSSIBLE MEANS OF DECREASING TRANSMISSION OF BRUCELLOSIS IN BISON

Lowell A. Miller,^{1,4} Jack C. Rhyan,² and Mark Drew³

¹ US Department of Agriculture-Animal Plant Health Inspection Service, National Wildlife Research Center, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA

² US Department of Agriculture-Animal Plant Health Inspection Service, Veterinary Services, National Wildlife Research Center, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA

³ Idaho Department of Fish and Game, 16569 S. 10th Ave., Caldwell, Idaho 83607, USA

⁴ Corresponding author (email: lowell.a.miller@usda.gov)

ABSTRACT: Preventing pregnancy in brucellosis-infected bison (*Bison bison*) provides a potential means of preventing transmission of disease. To determine whether a gonadotropin-releasing hormone (GnRH) vaccine was effective in reducing pregnancy in bison and to study the safety of injecting GnRH in pregnant bison, a study was conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho (USA). Four pregnant and two nonpregnant female bison were given a single injection of GnRH vaccine, and five pregnant adult females were given a sham injection that contained only adjuvant. Three of the GnRH-vaccinated bison that were pregnant at the time of vaccination delivered healthy calves. One treated bison had dystocia that resulted in a dead calf. All control bison delivered healthy calves. After calving, females of both groups were exposed to two bulls. Treated bison were palpated 6 wk after exposure to the bulls, and blood was drawn for pregnancy-specific protein B analysis. The six treated bison were not pregnant. The sham-treated bison became pregnant and delivered viable calves. This study demonstrates that a single dose of GnRH vaccine is effective in preventing pregnancy in female bison for at least 1 yr.

Key words: Gonadotropin-releasing hormone, immunocontraception, GnRH vaccine, bison.

INTRODUCTION

Bovine brucellosis, a bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*), and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or postparturient uterine discharge. Additionally, *Brucella* is shed in milk from infected dams and can be transmitted to calves through suckling. After initial infection, a dam often experiences abortion. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may also result in the shedding of *B. abortus*. The occurrence of venereal transmission of brucellosis in bison is unknown; however, on the basis of a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is considered unlikely to be a significant route of transmission. Transmission of the disease in cattle, bison, and elk, therefore, is primarily depen-

dent on the occurrence of pregnancy and exposure to abortion or calving in infected animals.

Rhyan et al. (2002) suggested that permanent sterilization, surgical or chemical, is a disease-management strategy that could be effectively used in *Brucella*-infected bison to greatly reduce the possibility of transmission to other animals. Bison cows could remain persistently infected with *B. abortus*, and, as long as the infected animals were not allowed to become pregnant, they would not be likely to transmit the infection. Therefore, disease prevalence might decrease dramatically as that generation of infected bison disappears. Objections have been raised to permanent sterilization in relation to wild horse immunocontraception, because it might result in the permanent removal of those animals from the gene pool and the creation of a new “unnatural” class of animals (Kirkpatrick and Turner, 1991).

The gonadotropin-releasing hormone

(GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns as the GnRH antibody titer drops below a protective level. This temporary period of infertility may allow time for *B. abortus* infection to clear.

The use of nonlethal methods to control populations of pest animals is an area of research that is receiving more interest (Fagerstone et al., 2002). Kirkpatrick et al. (1996) pioneered the use of porcine zona pellucida (PZP) for use as a nonlethal, contraceptive approach to pest animal control. The difficulty with the use of PZP in ungulates is that the animals that receive it, although they remain infertile, continue to have estrous cycles. Female white-tailed deer (*Odocoileus virginianus*) vaccinated with PZP have continued to exhibit sexual activity into February, 4 mo beyond the normal breeding season (Miller et al., 2000b). This continuous estrous cycling results in increased activity during early winter, at a time when the conservation of calories is important, although this increased cycling has not resulted in any apparent health problems (Miller et al., 2001). Additionally, it could increase the spread of venereally transmitted diseases, if present and, at least in the case of deer in populated areas, may contribute to increased collisions with automobiles. Prolonging the breeding season of bison in the greater Yellowstone area may be deleterious to the winter survival of dominant bulls and vaccinated cows because of increased activity during fall and early winter.

Immunocontraception using the GnRH vaccine is an alternative to PZP that would not extend the breeding season. The keyhole limpet hemocyanin–GnRH immunocontraceptive vaccine interferes with the release of follicle-stimulating hormone (FSH) and leutinizing hormone (LH), thereby preventing normal function of the ovaries and testes and their production of progesterone and testosterone. Thus, GnRH vaccine can effectively prevent

conception in either females or males (Talwar, 1985).

The GnRH vaccine has successfully produced sterility in Norway rats (*Rattus norvegicus*; Miller et al., 1997) and white-tailed deer (Miller et al., 2000a). The immunoneutralization of GnRH produces temporary nonsurgical castration in animals (Meloan et al., 1994; Oonk et al., 1998). In an ongoing study in female white-tailed deer conducted by the National Wildlife Research Center (Fort Collins, Colorado, USA) and Pennsylvania State University (University Park, Pennsylvania, USA), a single injection of GnRH vaccine resulted in infertility lasting up to 3 yr.

The development of immunocontraceptives that are practical to use for wildlife population control must include vaccine delivery systems. Although the administration of an oral form of the vaccine may be necessary in some situations, a long acting single-shot injectable form of the vaccine would have practical advantages over formulations that require two injections. Immunocontraception has typically required at least two doses, given as a prime and a boost. The prime dose prepares the immune system for a repeat antigen exposure and provides only a short-term immune response. The boost immunization can result in an immune response that may last for months to years. To have success with a single injection, the dose and timing of the injection is more critical than when using two injections. This article reports on the immunocontraception of penned bison using the newly developed single-shot GnRH vaccine.

MATERIALS AND METHODS

On 6 June 2002, six 6-yr-old female bison were injected with 1,800 µg of a single-shot GnRH vaccine (GonaCon/AdjuVac™, developed by the National Wildlife Research Center, United States Department of Agriculture, Animal Plant Health Inspection Service, Fort Collins, Colorado—patent pending) in a 1-ml injection given intramuscularly in the hip. Five control bison were injected with the adjuvant,

TABLE 1. Results of contraception in female bison using a GnRH vaccine.

Treatment	Year 1		Year 2		Calving dates, 2003
	Pregnancy status when injected (June 2002)	Calving dates, 2002	Pregnancy rate	PSPB ^a results	
Sham injection	5/5	20 June–26 July	5/5	5/5 positive for pregnancy	4 June–29 July
1,800 µg GnRH/AdjuVac ^b	4/6	28 June–1 July	0/6	0/6 positive for pregnancy	No calves born

^a PSPB = pregnancy-specific protein B.

^b GonaCon/AdjuVac, US Department of Agriculture, Animal Plant Health Inspection Service, patent pending.

1 ml, in the hip (control). All control bison and four of the treated bison were pregnant at the time of the injection. Because the GnRH vaccine has the potential to cause abortion, the pregnant bison were vaccinated to determine the safety of the GnRH vaccine. Blood samples were drawn monthly for 4 mo and then every other month for a total of 8 mo. Serum was tested for progesterone by radioimmunoassay and for GnRH antibody by enzyme-linked immunoassay (Miller et al., 2000a).

Two months after calving, a bull was introduced to the pen and allowed to breed the cows for 2.5 mo (17 September–1 December 2002). Six weeks after the bull was removed, both control and GnRH-treated bison were palpated for pregnancy diagnosis, and results were confirmed by serum pregnancy-specific protein B assay (PSPB) testing (Biotracking, Moscow, Idaho).

RESULTS

Analysis of pregnancy and calving data in the control and GnRH-treated bison at the time of GnRH injection and the following year indicated that the GnRH vaccine was successful in reducing reproduction, compared with controls (Table 1). At

the time of vaccination, five of the sham-treated cows and three of the six GnRH-treated cows were in the last month of pregnancy. Cows in both groups delivered normal calves the first year; therefore, the GnRH vaccine did not interfere with the pregnancy. None of the GnRH-treated cows became pregnant the year after the vaccination. All control bison conceived, and four had normal calves, with calving dates of 4–30 June 2003. One control cow died on 30 March 2003 but was pregnant at the time of death. During this study, two cows, one each in the treated and control groups, had dystocia that resulted in dead calves.

The average progesterone levels for pregnant cows were the same for the treatment and control groups at the start of the study and after calving. After rebreeding, the progesterone level of cows in the control group increased to pretreatment levels, indicating that they became pregnant, and anti-GnRH titers were not detected (Fig. 1). Progesterone levels in the GnRH-treated bison remained at nonpregnant levels (Fig. 2). All control bison delivered normal healthy calves and became pregnant again the second year. One of the five control bison died from accidental causes midgestation. The remaining four controls had normal calves in year 2 of the study.

Three of the six GnRH-treated bison were in late gestation when they were immunized, and all delivered normal calves within 1 mo after treatment. Two of the GnRH-treated cows were not pregnant at the time of GnRH vaccination, as suggest-

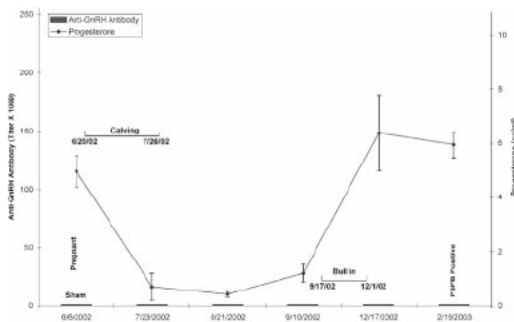


FIGURE 1. Average serum progesterone levels and anti-GnRH antibody titers for control bison cows.

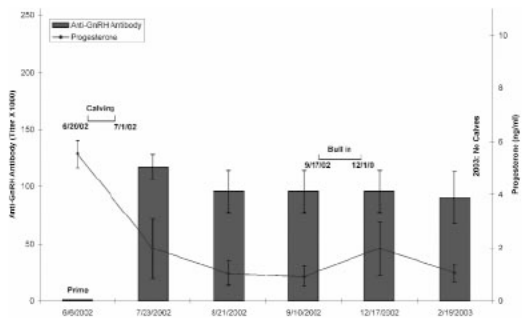


FIGURE 2. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated late during pregnancy.

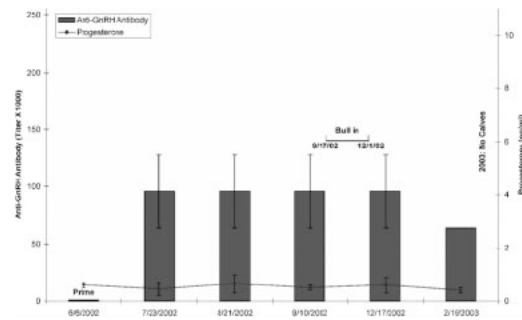


FIGURE 3. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated when not pregnant.

ed by low progesterone levels at the time of treatment (Fig. 3). The low progesterone values during the postpartum period in the cows that calved were comparable to those of the control cows. However, they did not become pregnant after exposure to the bulls, as indicated by low progesterone levels, the absence of a fetus on palpation, and negative PSPB serum test results.

An exception to the results of treatment of cows during late pregnancy was bison B-40, which had been given the GnRH vaccine during midpregnancy. This bison cow was injected with the vaccine on 6 June 2002 and delivered full term on 6 November 2002, with dystocia, resulting in a dead calf. The progesterone level progressively dropped from 7.0–3.5 $\mu\text{g}/\text{ml}$ of serum during the first 2 mo after vaccination and leveled off at 3.4 $\mu\text{g}/\text{ml}$ of serum during the third month, 2 mo before the birth of the dead full-term calf. This cow had a positive PSPB result, low progesterone levels, and was not pregnant on palpation.

The anti-GnRH data in treated bison indicated that a protective antibody titer was reached by the first time blood was collected (47 days after vaccination). The mean titer at this time was 112,000, decreasing to a mean titer of 72,000 by the end of the study. Antibody titers of 64,000 or greater have been shown to be consistent with contraception (Miller et al., 2000a).

In the beginning of the study, similar progesterone and PSPB levels in control and treated groups suggested that cows in both groups were pregnant. Calving dates were comparable in the control and treated cows, indicating synchronous breeding cycles (Table 1). However, in the second year, elevations in progesterone levels in the control group suggested that they became pregnant, and low progesterone levels in the treated group suggested infertility (Figs. 1–3).

DISCUSSION

The GnRH vaccine induces infertility in female mammals by reducing the release of FSH and LH, which, in turn, interferes with either the normal estrous or ovulatory cycle or reduces progesterone concentration during early pregnancy, which may interfere with maintenance of pregnancy. Stevenson (1997) stated that GnRH controls the amount of progesterone produced by the corpus luteum (CL), which maintains pregnancy for 200 days of the mean 280-day gestation in cattle. After 200 days, the ovary containing the CL can be removed without interfering with pregnancy, which indicates that pregnancy is not maintained by pituitary GnRH; the placenta apparently takes over the production and maintenance of progesterone. Bison have a gestation period similar to that of cattle.

Our results indicate that the GnRH vaccine can be administered safely during the

last third of pregnancy. Protective levels of anti-GnRH antibody require 30–45 days to develop, which suggests that the vaccine could be safely administered at ≥ 170 days of gestation without negative effects on the fetus. This was shown to be the case in the three bison treated late in pregnancy.

One cow was vaccinated during the second trimester of pregnancy and delivered a full-term dead calf on 6 November. It is unknown whether the GnRH vaccine contributed to death the fetus. There was a decrease in progesterone levels in this cow after vaccination that could have contributed to the loss of viability of the calf. Because the bull was with the cows from 17 September to 1 December, it is unlikely that this cow could have rebred. However, anti-GnRH antibody titers were sufficient in this cow to prevent pregnancy. One control bison also had a similar late full-term dead calf; thus, it is uncertain whether the vaccine caused the death of the calf in the vaccinated cow.

All control and treated cows were tested for pregnancy by palpation and serum progesterone and PSPB levels during February 2003. Bison B-40 had a positive serum PSPB test at this time but a low progesterone level and was not pregnant on palpation. Thus, the PSPB test was incorrect. This is consistent with reports that retained placentas following abortions can cause a false-positive PSPB result for several months (Sasser et al., 1986). The bison will be monitored for 2 more years to determine the duration of the contraceptive effect.

This study demonstrates that a single injection of GnRH vaccine is effective in preventing contraception in female bison for at least 1 yr. Booster injections lengthen the contraceptive effect in white-tailed deer (Miller and Killian, 2000), and lengthening the contraceptive effect in bison may be achieved similarly. Use of the GnRH vaccine in *Brucella*-infected bison should effectively reduce transmission of disease by reducing pregnancy rates and subsequent abortion or parturition.

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THE USE OF CONTRACEPTION AS A DISEASE MANAGEMENT TOOL IN WILDLIFE

Jack C. Rhyan, D.V.M, M.S., Lowell A. Miller, Ph.D., and Kathleen A. Fagerstone, Ph.D.

THE USE OF CONTRACEPTION AS A DISEASE MANAGEMENT TOOL IN WILDLIFE

Jack C. Rhyan, D.V.M, M.S., Lowell A. Miller, Ph.D., and Kathleen A. Fagerstone, Ph.D.

Abstract: Contraception offers potential as a tool for managing certain diseases in wildlife, most notably venereally transmitted diseases or diseases transmitted at parturition. Brucellosis is an excellent example of an infectious disease present in wild populations that could potentially be managed through immunocontraception. Previous studies in bison (*Bison bison*) suggest that a single injection of GonaCon™ (National Wildlife Research Center, U.S. Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services, Fort Collins, Colorado 80521, USA) results in 3 or more yr of infertility. Ongoing studies will determine if the use of GonaCon in bison decreases shedding of *Brucella abortus* from infected animals and will better define the duration of infertility following a single injection

Key words: Bison, *Brucella abortus*, brucellosis, gonadotropin releasing hormone, immunocontraception.

BRIEF COMMUNICATION

The management of diseases in wild populations presents many challenges, including those of difficult logistics, a paucity of efficacious techniques, effective vaccine or therapeutic delivery systems, and public acceptability. Contraception offers potential as a disease management tool for certain diseases, most notably venereally transmitted diseases or diseases transmitted at parturition. Brucellosis, a group of zoonotic diseases caused by bacteria in the genus *Brucella*, is an excellent example of a group of infections present in wild populations that could potentially be managed through immunocontraception. Swine brucellosis, caused by *Brucella suis*, is transmitted through the venereal route as well as through contact with aborted fetuses and placental membranes and fluids. Bovine brucellosis, caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*), and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or postparturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The

occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison and studies in cattle, it is not considered likely to be a significant route of transmission.^{1,4,5} Therefore, transmission of disease in cattle, bison, and elk is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™ (National Wildlife Research Center, U.S. Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services, Fort Collins, Colorado 80521, USA), a gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine, is approved for use in wild white-tailed deer (*Odocoileus virginianus*), in which a single injection usually results in 2 or more yr of infertility.² This study reports the results of three small pilot studies examining the use of GonaCon to prevent parturition in bison.

The first study was conducted at Northwest Trek, a zoologic park in the state of Washington (USA). Its purpose was to determine if the GnRH vaccine had any effect on the reproductive success of five breeding-age female bison (age range: 2–8 yr) as compared to the herd's normal reproductive history of over 60% reproduction annually. The five bison received 1,800 µg of the vaccine by intramuscular injection between 21 May and 26 July 2001. The animals were continuously exposed to multiple bulls. Three of the five did not calve in 2002, one had a live calf, and one died because of dystocia. Because of the incomplete contraception of the group, the remaining four bison were boosted with the same dose of vaccine on 30 August 2002. The boosted bison did not calve in 2003, 2004, or 2005. By 2012, at least two of the three bison remaining alive had given birth to one or more calves. These results suggested a

From the United States Department of Agriculture, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, Colorado 80521, USA (Rhyan, Miller, Fagerstone). Correspondence should be directed to Dr. Rhyan (jack.c.rhyan@aphis.usda.gov). The authors prepared this article as part of their official duties with the U.S. Government, and therefore unable to assign rights to the American Association of Zoo Veterinarians.

Table 1. Results of pilot study comparing the reproductive results per year of single dose gonadotropin releasing hormone vaccinated bison (1,800 µg) with nonvaccinated controls.

Treatment group	2002 ^a	2003	2004	2005	2006	2007	2008	Total calves/RYS ^b
Controls	5/5	4/5	4/4	1/3	2/3	3/3	2/3	16/21
Vaccinates	4/6	0/6	0/6	0/5	0/5	0/5	0/5	0/32

^a 4-yr-old bison were vaccinated in June 2002. 4 of 6 were in mid or late pregnancy when vaccinated.
^b Total number of calves produced per reproductive years of cows in the study.

high degree of vaccine efficacy in bison following two vaccinations.

The second study, conducted from 2002 until 2008, was begun at the Idaho Department of Fish and Game wildlife research facility in Caldwell, Idaho (USA). The study utilized bison that were offspring of animals that had been trapped at the border of Yellowstone National Park in 1997 and taken to the Idaho facility for study. In 2004, the bison were moved to the Colorado State University, Animal Population Health Institute’s wildlife research facility in Fort Collins, Colorado (USA). The bison were serologically negative for brucellosis throughout the study. This study compared reproductive results of six bison intramuscularly vaccinated with a single dose of 1,800 µg GnRH on 06 June 2002 to those of five sham-vaccinated controls that received the adjuvant only. Breeding season for bison usually begins in July and may continue for several months. Results of the first year of this study have been previously reported.³ Four of the vaccinees were in mid- or late-term pregnancy when vaccinated. Results (Table 1) indicate that the vaccine did not interfere with reproductive success of animals in mid- and late-term pregnancy at the time the vaccine was administered. More importantly, a single dose of

vaccine resulted in infertility in all vaccinees for the duration of the study.

The third study, conducted from 2003 until 2008, evaluated the efficacy of GonaCon in bison at low, medium, and high doses (1,000, 2,000, and 3,000 µg, respectively). That study was conducted on bison purchased from a producer and was begun at a private ranch in Gardiner, Montana (USA). Bison were vaccinated on 20 May 2003; controls received the adjuvant only. After 2 yr, animals were moved to a private ranch in eastern South Dakota (USA). The first year of the study (2003), female bison were pastured with a bull that was later discovered to be infertile; therefore, the first exposure of the study animals to a fertile bull was summer 2004, 14 mo after vaccination. Results of that study (Table 2) indicate the vaccine had increased efficacy at the higher dose, resulting in 3 yr of infertility in three of four bison.

Two ongoing studies are designed to 1) evaluate the duration of infertility in 10 vaccinated bison that received 3,000 µg GonaCon as compared to 10 controls in a range setting; and 2) determine if the use of GonaCon decreases shedding of *B. abortus* from a group of 14 naturally infected bison during the calving season as compared to a similar group of nonvaccinees. If these studies confirm the safety and efficacy of GonaCon in bison and demonstrate its utility in reducing shedding of *B. abortus*, the vaccine could provide a potential nonlethal management tool to prevent transmission of the disease in an infected bison population.

Table 2. Results of dose response study comparing reproductive results per year of nonvaccinated controls and single shot low (1,000 µg), medium (2,000 µg), and high (3,000 µg) doses of gonadotropin releasing hormone vaccine in bison.

Treatment	2004 ^a	2005	2006	2007	2008	Calves/RYS ^b
Controls	0/5	5/5	4/5	2/3	ND ^c	11/13
Low dose	0/5	2/5	3/5	2/4	ND	7/14
Medium dose	0/5	3/5	2/5	0/2	0/1	5/13
High dose	0/4	0/4	1/4	1/4	0/2	2/14

^a 2-yr-old female bison were vaccinated in May 2003 and were placed with breeding bull in July 2004.
^b Total number of calves produced per reproductive years of cows in the study.
^c ND not done.

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From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: RE: WiLDIT Accomplishments
Date: Tuesday, October 06, 2015 8:37:00 AM
Attachments: [WiLDIT Accomplishments FY 2015 PN additions.docx](#)

Here's what I've added. Probably have forgotten a few things...

From: Rhyan, Jack C - APHIS
Sent: Monday, October 05, 2015 4:02 PM
To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS
Subject: WiLDIT Accomplishments

Hey,

Suelee needs WiLDIT accomplishments. She said we can just list them corporately. I gave it a start and would appreciate you all helping out. Matt, I included the bison transfer but feel free to rewrite it as you wish. Pauline, it needs all your feral swine work and VOCs and papers, talks, etc.

Thanks much

Jack

WiLDIT Accomplishments FY 2015

Bison and elk work:

Continued GonaCon studies in southern Colorado and Montana

Assisted CSU in reproductive work

Played key role in preparations for release of YNP genetics bison on public ground to found the Laramie Foothills Conservation Herd (Nov 1 is release date) and arranged to provide bulls with YNP genetics for founder herd at Midewyn Tall Grass Prairie in Illinois. (Relocation occurs mid-October)

Currently conducting challenge study using finely powdered RB51 compounded with montmorilite in mice. If successful, we will try this approach in elk.

Submitted work for patent on DryDart to ARS patent committee. ARS patent committee accepted assignment and submitted application to Patent office. We are continuing to develop DryDart and are starting live animal study to measure immune response to RB51 delivered by DryDart compared to hand vaccination.

Arranged with the states of Colorado and Wyoming to collect up to 20 *Brucella*-positive, pregnant, wild elk cows at feedgrounds in Wyoming in winter 2016, to be transported to Colorado for a natural *Brucella* transmission study.

Conducted study in collaboration with Colorado Parks and Wildlife and Wyoming Game and Fish Commission on efficacy of a Nalbuphine/Azaperone/Medetomidine drug combination in bison.

Feral Swine:

Continue vaccine study investigating efficacy of killed oral *Mycobacterium bovis* (Spanish and Michigan strains) in feral swine of Texas origin.

Participated in feral swine ear tag study to determine feasible ear tag weights in the context of eventual application of satellite ear tags.

Investigation of use of volatile organic compounds in breath and feces of swine for detection of *Mycobacterium tuberculosis* complex infection. Collected breath and fecal VOCs from wild boar in Doñana National Park in Spain, September 2015, to be analyzed by collaborators at Roviri i Virgili University in Tarragona, Spain. Will collect VOCs from feral swine in an experimental *M. bovis* challenge in Fall, 2015, also to be analyzed by Roviri i Virgili University.

Visited Texas A and M facility in Kingsville, TX to explore possibilities in collaborative feral swine work with researchers at that university.

Working with Hawaii Department of Agriculture to receive feral swine from Molokai, HI for future testing of tuberculosis vaccines.

Cattle

Collected breath and fecal samples from Michigan dairy cattle involved in an outbreak of *M. bovis*. Samples were sent to the Technion, Haifa, Israel for volatile organic compound analysis.

Publications and presentations:

Rhyan JC, Tyers D, Zimmer J, Lewandowski K, Hennager S, Young J, Pappert R, Panella A, and Kosoy, O. 2015. Serologic survey of snowshoe hares in the Greater Yellowstone Area for brucellosis, tularemia, and snowshoe hare virus. *J Wildl Dis* 51:769-773.

USAHA Brucellosis Scientific Subcommittee, Research Updates. October 19, 2014.

Presented Research Updates to National Academy of Sciences, Brucellosis Review Panel September 15, 2015

Presented Research Updates to Brucellosis Research Group in Jackson, WY, Sept 24, 2015.

Stahl R.S., Ellis C.K., **P. Nol**, W.R. Waters, M. Palmer, and K.C. VerCauteren. 2015. Fecal volatile organic compound profiles from white-tailed deer (*Odocoileus virginianus*) as indicators of *Mycobacterium bovis* exposure or *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) vaccination. PLoS One 10(6):e0129740.

Fagre, A., K.A. Patyk, **P. Nol**, T. Atwood, K. Hueffer, and C. Duncan. 2015. A review of infectious agents in polar bears (*Ursus maritimus*) and their long-term ecological relevance. Ecohealth. 2015 Mar 20.

Patyk, K.A., C. Duncan, **P. Nol**, C. Sonne, K. Laidre, M. Obbard, Ø Wiig, J. Aars, E. Regehr, L.L. Gustafson, and T. Atwood. Establishing a definition of polar bear (*Ursus maritimus*) health: a guide to research and management activities. 2015. Sci Total Environ. 514:371-8.

Presented update on wild swine tuberculosis on an international scale and participated in a panel discussion. Many Hosts of Mycobacteria VI: Host Specificity and Dynamics of Mycobacterial Disease, March 26-27, 2015, Tulane National Primate Research Center, Covington, Louisiana,

Presented to undergraduate and graduate students on tuberculosis at epidemiology course at Colorado State University

Presented to students and faculty at the Department of Electronics, Electrical and Automatic Engineering at Rovira i Virgili University, Tarragona, Spain on WiLDIT volatile organic compound research.

Students/Externs:

Eight students were accepted as veterinary externs in FY15. These externs were hosted at NWRC by WiLDIT for two to four week blocks. They represented five veterinary schools in the country. One MPH/veterinary student at Colorado State University was co-mentored over the summer by NWRC researchers and WiLDIT researchers. One local high school student interned for WiLDIT over the summer.

WiLDIT has one Saul T. Wilson Scholar from Colorado State University. This student is participating in lyophilized *Brucella* vaccine research in mice.

WiLDIT is supporting and mentoring one MS student at Colorado State University. This student is a DVM and is participating in feral swine tuberculosis vaccine research.

Served on PhD committee for DVM/PhD student studying brucellosis at Colorado State University.

Five undergraduate students from Colorado State University perform animal care and maintenance at the WiLDIT wildlife research facility. These students are supported through a cooperative agreement with the Animal Population Health Institute at Colorado State University.

Other:

Two WiLDIT members participated in HPAI task force in Minnesota, May 3–June 1.

Two WiLDIT members participated in a regulatory veterinary medicine laboratory at Colorado State University.

Produced a material transfer agreement with NEIKER, Spain to use killed *Mycobacterium bovis* vaccine in feral swine.

Established cooperative agreement with the Department of Electronics, Electrical and Automatic Engineering, Rovira i Virgili University, Tarragona, Spain to conduct volatile organic compound analysis on breath and feces to detect tuberculosis in wild boar and feral swine

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to conduct tuberculosis volatile organic compound and molecular research, and support the wildlife research facility.

Established cooperative agreement with the Department of Pathobiological Sciences to conduct tuberculosis vaccine research in feral swine.

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to support a MS student in research of tuberculosis vaccines in feral swine.

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: Recap....10/6/15 call
Date: Tuesday, October 06, 2015 3:26:08 PM

Just thought I would put this down in writing for all of us.....you can tell me if I got it wrong!

Check Jan 12-14 for preg check/transmitter placement on GonaCon animals.

We will do our best to keep all of the animals separate at Hunters, and have only controls at Hunters pens starting in January. This will lessen the risk to the study if there is a breach at the Corwin pens and allow us to keep the original control group through 5 birth events.

Look into paneling critical areas of Hunters corrals and replacing gates in order to make them last until end of this study at minimum.

Becky will not be the last GYA employee standing.....

From: [Nol, Pauline - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: Renewal for GonaCon study
Date: Thursday, December 12, 2013 4:07:00 PM
Attachments: [Study Protocol Renewal GonaDon for Brucella Management in Bison Dec 2013-JRhyan.pdf](#)

Hey there Ryan,

I've attached the protocol renewal for the GonaCon study to be evaluated by the Quarantine IACUC.
Let me know if the information is (not) adequate.

Thanks!

Pauline

Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

2. Action needed:

 Project Completed

 Project Never Initiated

 X Project On-going/Active: renew as approved

 Project On-going/Active: renew with minor revisions

 Project Not Yet Initiated or is Inactive: renewal requested

Anticipated start date

3. Protocol Changes

In the upcoming year will you implement any changes to the animal component of the project that differ from those in the original (or subsequent) approval by the IACUC (e.g. changes to animal procedures, number of animals needed, or project objectives)?

Yes No X

3. Animal Use and Procedure alternatives since the last IACUC approval

a. Have alternatives to the use of animals become available that could be substituted to achieve specific project aims? Yes No X

b. Have alternatives that are potentially less painful or distressful to animals become available that could be used to achieve specific project aims? Yes No X

If you answered yes to either question, please provide a description below or attach one.

N/A

Animal usage (please complete the following box):

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved FOR TOTAL PROJECT on current approval notification <u>plus</u> any subsequent amendments	104			
2. Number of animals used during first IACUC approval year	40			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	40			
4. Number of animals used during third approval year	42			

Note: Additional animals (up to 62) will be collected in winter/spring 2014 to replicate the study as described in the original protocol.

Study Director



Date 12/12/13

Concur

IACUC Chair _____

Date _____

From: [Clarke, Patrick R. - APHIS](#)
To: [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Ahola, Sara C - APHIS](#)
Subject: research planning meeting
Date: Thursday, November 17, 2016 1:48:19 PM

All,

Becky and I will be making a road trip down to Laramie and Ft Collins on December 13-14. We were hoping we could all set aside the morning of December 14th to have a research planning meeting at NWRC (or Bldg B?). Specifically we want have a frank discussion about future research at the Montana facility....the strength of the proposals, priority of the proposals, the animal sources, rolling up the GonaCon study in relation starting other studies, disposition of animals, linkages with ongoing NWRC research, etc. This might take more than just a morning, but for planning sake I hope we can at least plan on being together from 8 am-12 pm. Will this work for everyone?

Ryan

P. Ryan Clarke, DVM, MPH
USDA, APHIS, VS, SPRS
District 5 Epidemiologist-GYA
Bozeman, Montana
406-388-5162

From: [Clarke, Patrick R. - APHIS](#)
To: [Herriott, Donald E - APHIS](#); [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Ahola, Sara C - APHIS](#)
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P. Ryan Clarke, DVM, MPH
USDA, APHIS, VS, SPRS
District 5 Epidemiologist-GYA
Bozeman, Montana
406-388-5162

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: sample list
Date: Tuesday, January 27, 2015 3:10:20 PM

Hi,

So when the Red 52 calves.....and her implant is 151.134.....we are taking blood, serum and whole; feces; vaginal swab; any fluid or placenta; milk, all four quarters into one tube; conjunctival swab, and blood from calf.....or submit the abortion. All within 5 days of birth. Usually within 3 days though.....The swabs go into 1 ml WHO. All samples need quantification from NVSL. I don't know how long you will keep her, but those are the samples we will need to compare her to the rest of the group one day.

Thanks!

Becky

From: [Rhyan, Jack C - APHIS](#)
To: [Ellis, Robert \(b\) \(6\) @ColoState.EDU](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: select agent vs natural infection
Date: Friday, June 14, 2013 4:01:34 PM

Bob,
Down the string a ways you will see the email from Freeda Isaac that is golden.
Jack

-----Original Message-----

From: Quance, Christine R (APHIS)
Sent: Wednesday, August 17, 2011 8:19 AM
To: Rhyan, Jack C (APHIS); Robbe Austerman, Suelee (APHIS); Henry, Lisa A (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: RE: seropositive brucella cows in Idaho

Hi Jack,
Thanks for sending this. We are registered with the CDC now instead of APHIS, but they should have the same rules.
I'll send a separate email on the media.

Chris Quance
Microbiologist-Team Leader, Mycobacteria and Brucella Section National Veterinary Services Laboratory
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.R.Quance@aphis.usda.gov

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-----Original Message-----

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, August 16, 2011 5:30 PM
To: Quance, Christine R (APHIS)
Cc: Frey, Rebecca K (APHIS); Nol, Pauline (APHIS); McCollum, Matthew P (APHIS)
Subject: FW: seropositive brucella cows in Idaho

Chris,
See below the info from Freeda that says we now can sample a naturally infected animal time after time and the specimen is the select agent once it is confirmed positive. We will be doing this both with some seropositive bison bulls soon and starting next spring with the contraception study. I'll give you a call and talk about the bull study. Also, who should I talk to about getting some fresh WHO media in tubes?
Jack

-----Original Message-----

From: Freeda E Isaac [<mailto:freeda.e.isaac@aphis.usda.gov>]
Sent: Thursday, May 13, 2010 11:50 AM
To: Jack C Rhyan
Subject: Fw: seropositive brucella cows in Idaho

Hi Jack,

As we had discussed over the phone several weeks ago, the Select Agent Program directors were meeting to discuss the issues related to naturally infected versus experimentally infected animals and the status of samples taken from these animals.

In our discussions, it was agreed upon that for naturally affected animals, samples taken from those animals would not be considered select agent material and required to be handled as restricted material until the sample was confirmed to have select agent material. For the issues you have raised below for the cattle you have, the samples may be handled as you have described and not subject to select agent requirements until the sample itself is confirmed positive for select agents.

For your questions regarding registration with the select agent program, you would need to have a security risk assessment completed which is a background check by FBI. That would be different than what would have been done at Plum Island previous to about 2005. I will give you a call to discuss other issues with the cattle. For information on completing the registration documents, you can call Sherylyn Roberson at 301-734-5460.

Thanks, Freeda

Freeda E. Isaac, DVM

Director

National Center for Import Export

USDA/APHIS/Veterinary Services

Phone: 301-734-8364

Fax: 301-734-6402

Email: Freeda.E.Isaac@aphis.usda.gov

----- Forwarded by Freeda E Isaac/MD/APHIS/USDA on 05/13/2010 01:35 PM -----

Freeda E Isaac/MD/APHIS/USDA

04/02/2010 12:14 PM

To

Jack C Rhyan/CO/APHIS/USDA@USDA

cc

Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Matt

McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject

Re: seropositive brucella cows in Idaho

Hi Jack,

Although naturally infected animals are not considered select agents themselves and not subject to the select agent regulations, once these animals are confirmed as positive for a select agent, any materials from these animals would be treated as select agent material. The infected cattle are considered the natural source of the Brucella and the materials from these animals are being intentionally collected. This is found in 9 CFR 121.3(d)(1). For example, blood, tissue specimens, urine, etc. would be subject to handling as select agent material. My understanding is these Idaho cattle have been confirmed for B. abortus by VS, therefore the materials from these cattle would need to be handled in accordance with the select agent requirements.

9 CFR 121.6(a)(1) requires that specimens are transferred to a select agent registered facility for that particular select agent within 7 calendar days. 9 CFR 121.16 describes the transfer process in which a APHIS/CDC Form 2 is completed and submitted to APHIS for approval prior to the transfer.

Let me know if you have any other questions. Freeda

Freeda E. Isaac, DVM
Director
National Center for Import Export
USDA/APHIS/Veterinary Services
Phone: 301-734-8364
Fax: 301-734-6402
Email: Freeda.E.Isaac@aphis.usda.gov

Jack C Rhyan/CO/APHIS/USDA
03/22/2010 05:44 PM
To
Freeda E Isaac/MD/APHIS/USDA@USDA

cc
Cynthia M Gaborick/ID/APHIS/USDA@USDA, Mary K Tinker/ID/APHIS/USDA@USDA, Pauline
Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA

Subject
seropositive brucella cows in Idaho

Freeda,
Current situation: Idaho has 4 seroconverters, at least 2 have aborted. Milk and fetus from one cow has been submitted for culture.
My proposal: I would purchase the 4 and bring to Fort Collins (with state vet's and AVIC's approval). We would then collect large amounts of blood for NVSL's serum bank. We would periodically collect urine from them; filter it to exclude bacteria and submit urine samples for GC/mass spec analysis for volatile organic compounds (VOCs) specific for brucella. We would also analyze breath for VOCs. Breath analysis and urine can be done here. Urine also might be shipped to APHIS personnel at a laboratory in PA for GC/MS analysis. I think this project would last 6 months at the end of which we would kill cows and submit specimens for culture.

Please advise me on this potential study in view of select agent requirements.
Thanks much for your help.
Jack

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: serum in bank
Date: Friday, March 06, 2015 7:22:22 AM

Hi all,

Ryan and I were sweating for hours, poor conditions, no lunch, wading in stacks and stacks of old serum yesterday. As it turns out, we have A LOT of BQFS serum taking up more space in our shared -70 than we are supposed to use. I asked Matt about shipping some or all of it down to you, and then it occurred to me that maybe we don't need to keep stack and stacks of negative serum from the same animals. What do you all think about A) keeping it in CO, and B) dumping some? If so, we need to consider which may be the most valuable to keep. Keeping in mind we still have access to almost all of these animals at Fort Peck for the next 3 years as well. Does anybody know of somebody that could use it? Ie.....looking at other interesting things in the serum of YNP origin bison? RB51 vaccinate study, mycoplasma....

That's my pitch.

Jack, any ideas on where you would like to go with whole blood culture yet?

Thanks

Rebecca Frey
Wildlife Biologist/Disease Specialist
USDA APHIS VS
Montana
406-333-4425 office/fax

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: serum is shipped. 3 boxes
Date: Tuesday, March 10, 2015 2:43:19 PM

The GonaCon titer bag of serum is in one of the boxes. I am faxing the list of serum/ID's in the bag....after I get a new phone cord ☺ The rest are small boxes of 1st cohort BQFS serum. We ran out of boxes for the tall serum tubes, but I think that you now have all of the 1st cohort serum. It is labeled kinda of every which way.....because 3 different people stored it....over the years. Lucky for us....I have all of the original test charts, and the database in order to figure out what is what, as many are labeled by case number of the lab. They are all labeled for 1st cohort and a date though. We still have to finish boxing up the 2nd cohort, and when we get done will know how much more room we need to create in our freezer.....and may need to ship down some more to you. It doesn't hardly look like we did anything today!!!

Thanks

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: spreadsheet
Date: Tuesday, September 27, 2016 9:17:43 AM
Attachments: [Jack_Pregnancy.xlsx](#)

REBECCA FREY

Wildlife Biologist

USDA APHIS VS

Montana

406-333-4425

Bangle Tag	GonaCon	Pregnant 2013	Birth	Serology	Culture	Pregnant 2014
CONTROL 1						
Green 08	No	Yes	Calf	Neg	Neg	Yes
Green 09	No	Yes	Weak Calf	Pos	Pos	No
Green 10	No	Yes	Weak Calf/Abortion	Pos	Pos	Yes
Green 14	No	Yes	Calf	Neg	Neg	Yes
Green 15	No	Yes	Abortion (mummy)	Pos	Neg	Yes
Red 03	No	Yes	Abortion	Pos	Pos	Yes
Red 06	No	Yes	None, removed radio	Pos	Neg	Yes
Red 07	No	Yes	Calf	Pos	Neg	Yes
Red 08	No	Yes	Calf	Pos	Neg	Yes
Red 09	No	No	n/a	Pos	Neg	No
Red 13	No	Yes	Calf	Pos	Pos	Yes
Red 15	No	No	n/a	Pos	Neg	Yes/Dead
Red 16	No	Yes	Abortion	Pos	Pos	Yes
Red 17	No	No	n/a	Pos	Neg	No
Red 18	No	Yes	Calf	Pos	Neg	No
Red 20	Yes	Yes	Calf	Pos	Neg	No
Red 21	No	Yes	Calf	Pos	Pos	Yes
Red 22	No	Yes	Calf	Pos	Neg	Yes
Red 24	Yes	Yes	Calf	Pos	Neg	Yes
Red 25	No	Yes	Calf	Pos	Neg	Yes
Red 26	Yes	Yes	Calf	Pos	Neg	No
Red 30	No	Yes	Calf	Pos	Neg	DEAD
GONACON PEN 1						
Green 02	No	Yes	Calf	Neg	Neg	Yes
Green 03	No	Yes	Calf	Neg	Neg	No
Green 04	No	No	n/a	Neg	Neg	Yes
Green 06	No	No	n/a	Sus	Neg	Yes
Green 17	No	Yes	Calf	Neg	Neg	Yes
Red 01	Yes	No	n/a	Neg	Neg	No
Red 02	Yes	No	n/a	Pos	Neg	Yes
Red 04	Yes	No	n/a	Neg	Neg	No
Red 05	Yes	No	n/a	Pos	Neg	No
Red 11	Yes	No	n/a	Neg	Neg	No
Red 14	Yes	No	n/a	Pos	Neg	No
Red 19	Yes	No	n/a	Pos	Neg	No
Red 23	Yes	No	n/a	Pos	Neg	No/Dead
Red 27	Yes	No	n/a	Neg	Neg	No
Red 28	Yes	No	n/a	Pos	Neg	No
Red 29	Yes	No	n/a	Pos	Neg	No
Red 31	Yes	No	n/a	Neg	Neg	No
GONACON PEN 2						
Green 01						
Green 07						

Bangle Tag	GonaCon	Pregnant 2013	Birth	Serology	Culture	Pregnant 2014
Green 13						
Green 21						
Green 24						
Green 25						
Red 32						
Red 34						
Red 36						
Red 38						
Red 39						
Red 41						
Red 42						
Red 43						
Red 44						
Red 45						
Red 46						
Red 47						
Red 48						
Red 49						
Red 51						
Red 52				Shipped to CO 2015		
Red 53						
Red 54						
Red 55						
Red 56						
Control Pen 2						
Green 11						
Green 12						
Green 18						
Green 20						
Green 30						
3G17						
3G08						
3G14						
3R20						
3R22						
Red 33						
Red 35						
Red 37						
Red 40						
Red 50						
Red 64						
Red 67						
Red 68		Died 1/13/16-trauma in pens				
Red 71						
Red 73						
Red 74						

Birth	Serology	Culture	Pregnant 2015	Birth	Serology	Culture
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a		neg	Yes	Calf	Pos	Neg
Abortion	Pos	Neg/strep. Uberis	Yes	Mummy/Cow Died 5/18/15	Pos	Neg
Abortion	Pos	Pos	Yes	Abortion	Pos	Pos
Abortion	Pos	Pos	Yes	Calf	Pos	Neg
Calf	Pos	Pos	Yes	Calf	Pos	Pos
Calf	Pos	neg	Yes	Abortion	Pos	Neg
Calf	Sus	neg	Yes	Calf	Neg	Neg
Abortion	Pos	Pos	Yes	Abortion	Pos	Pos
n/a	Pos	neg	Yes	Stillbirth	Pos	Pos
Calf	Pos	Neg	Yes	Calf	Pos	Neg
DEAD		DEAD-No samples				
Calf	Pos	neg	Yes	Calf	Pos	Neg
n/a	Neg	neg	No	n/a	Pos	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
n/a	Pos	neg	Yes	Abortion	Pos	Neg
Calf	Pos	neg	Yes	Abortion	Pos	Pos
Calf	Pos	neg	Yes	Calf	Pos	Neg
Calf	Pos	neg	Yes	Calf	Pos	Neg
resorb/abortion?	Pos	Neg	No	n/a	Pos	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
DEAD		DEAD-No isolation on Retro or Mammary				
Calf	Neg	neg	Yes	Abortion	Neg	Neg
n/a	Neg	neg	Yes	Calf	Neg	Neg
Calf/Dead	Neg	neg	Yes	Calf	Neg	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a	Pos	neg	No	n/a	Sus	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Neg	neg	No	n/a	Neg	Neg
n/a	Pos	neg	No	n/a	Neg	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
DEAD		DEAD	B. abortus BV1; retro LN at death			
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Neg	neg	No	n/a	Neg	Neg
			Yes	Calf	Neg	Neg
			Yes	Stillbirth/huge calf	Neg	Neg

Birth	Serology	Culture	Pregnant 2015	Birth	Serology	Culture
			Yes	Calf	Neg	Neg
			No	n/a	Neg	Neg
			No	n/a	Neg	Neg
			Yes	Calf	Neg	Neg
			No	n/a	Neg	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			Yes	Calf	Pos	
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
			No	n/a	Pos	Neg
		Postmortem B abortus Positive culture				

Pregnant 2016	Birth	Serology	Culture
Yes?	No	Pos	
Yes	Dead calf/breach Euthan. 6/8/16	Pos	Neg
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
Yes	Calf	Pos	Pos
Yes	Calf	Neg	Neg
Yes	Calf	Pos	Neg
Yes	Calf-early/weak	Pos	Pos
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	
No	Shipped to CO		
Yes	Calf	Pos	Neg
Yes?	No	Pos	
Yes	Calf	Pos	Pos
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
No			
Yes	Calf	Pos	
Yes	Calf	Neg	Neg
Yes	Stillborn	Neg	Neg
Yes	Calf	Neg	Neg
Yes	Abortion	Neg	Neg
Yes	Calf	Neg	
No	n/a	Pos	
Yes	Calf	Neg	
no	n/a	Pos	
no	n/a	Neg	
no	n/a	Neg	
no	n/a	Sus	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Neg	
Yes	Calf	Neg	Neg
Yes	Calf	Neg	Neg

Pregnant 2016	Birth	Serology	Culture
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
No	n/a	Pos	
Yes	Calf	Pos	Neg
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Abortion	Pos	Pos
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Neg	Neg
Yes	Abortion	Pos	Pos
Yes	Stillbirth/breach	Neg	Neg
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
Yes	Calf	Neg	
Yes	Calf	Pos	Pos
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	Neg
Yes	Calf	Pos	Neg
No	n/a		
Yes	Calf	Pos	
No	n/a		
No	n/a		
No	n/a		
No	n/a		
No	n/a		
No	n/a		
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
No	n/a		

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: spreadsheet
Date: Tuesday, September 27, 2016 9:17:43 AM
Attachments: [Jack_Pregnancy.xlsx](#)

REBECCA FREY

Wildlife Biologist

USDA APHIS VS

Montana

406-333-4425

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Green 14	No	Yes	Calf	Neg	Neg	Yes
Green 15	No	Yes	Abortion (mummy)	Pos	Neg	Yes
Red 03	No	Yes	Abortion	Pos	Pos	Yes
Red 06	No	Yes	None, removed radio	Pos	Neg	Yes
Red 07	No	Yes	Calf	Pos	Neg	Yes
Red 08	No	Yes	Calf	Pos	Neg	Yes
Red 09	No	No	n/a	Pos	Neg	No
Red 13	No	Yes	Calf	Pos	Pos	Yes
Red 15	No	No	n/a	Pos	Neg	Yes/Dead
Red 16	No	Yes	Abortion	Pos	Pos	Yes
Red 17	No	No	n/a	Pos	Neg	No
Red 18	No	Yes	Calf	Pos	Neg	No
Red 20	Yes	Yes	Calf	Pos	Neg	No
Red 21	No	Yes	Calf	Pos	Pos	Yes
Red 22	No	Yes	Calf	Pos	Neg	Yes
Red 24	Yes	Yes	Calf	Pos	Neg	Yes
Red 25	No	Yes	Calf	Pos	Neg	Yes
Red 26	Yes	Yes	Calf	Pos	Neg	No
Red 30	No	Yes	Calf	Pos	Neg	DEAD
GONACON PEN 1						
Green 02	No	Yes	Calf	Neg	Neg	Yes
Green 03	No	Yes	Calf	Neg	Neg	No
Green 04	No	No	n/a	Neg	Neg	Yes
Green 06	No	No	n/a	Sus	Neg	Yes
Green 17	No	Yes	Calf	Neg	Neg	Yes
Red 01	Yes	No	n/a	Neg	Neg	No
Red 02	Yes	No	n/a	Pos	Neg	Yes
Red 04	Yes	No	n/a	Neg	Neg	No
Red 05	Yes	No	n/a	Pos	Neg	No
Red 11	Yes	No	n/a	Neg	Neg	No
Red 14	Yes	No	n/a	Pos	Neg	No
Red 19	Yes	No	n/a	Pos	Neg	No
Red 23	Yes	No	n/a	Pos	Neg	No/Dead
Red 27	Yes	No	n/a	Neg	Neg	No
Red 28	Yes	No	n/a	Pos	Neg	No
Red 29	Yes	No	n/a	Pos	Neg	No
Red 31	Yes	No	n/a	Neg	Neg	No
GONACON PEN 2						
Green 01						
Green 07						

Bangle Tag	GonaCon	Pregnant 2013	Birth	Serology	Culture	Pregnant 2014
Green 13						
Green 21						
Green 24						
Green 25						
Red 32						
Red 34						
Red 36						
Red 38						
Red 39						
Red 41						
Red 42						
Red 43						
Red 44						
Red 45						
Red 46						
Red 47						
Red 48						
Red 49						
Red 51						
Red 52					Shipped to CO 2015	
Red 53						
Red 54						
Red 55						
Red 56						
Control Pen 2						
Green 11						
Green 12						
Green 18						
Green 20						
Green 30						
3G17						
3G08						
3G14						
3R20						
3R22						
Red 33						
Red 35						
Red 37						
Red 40						
Red 50						
Red 64						
Red 67						
Red 68			Died 1/13/16-trauma in pens			
Red 71						
Red 73						
Red 74						

Birth	Serology	Culture	Pregnant 2015	Birth	Serology	Culture
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a		neg	Yes	Calf	Pos	Neg
Abortion	Pos	Neg/strep. Uberis	Yes	Mummy/Cow Died 5/18/15	Pos	Neg
Abortion	Pos	Pos	Yes	Abortion	Pos	Pos
Abortion	Pos	Pos	Yes	Calf	Pos	Neg
Calf	Pos	Pos	Yes	Calf	Pos	Pos
Calf	Pos	neg	Yes	Abortion	Pos	Neg
Calf	Sus	neg	Yes	Calf	Neg	Neg
Abortion	Pos	Pos	Yes	Abortion	Pos	Pos
n/a	Pos	neg	Yes	Stillbirth	Pos	Pos
Calf	Pos	Neg	Yes	Calf	Pos	Neg
DEAD		DEAD-No samples				
Calf	Pos	neg	Yes	Calf	Pos	Neg
n/a	Neg	neg	No	n/a	Pos	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
n/a	Pos	neg	Yes	Abortion	Pos	Neg
Calf	Pos	neg	Yes	Abortion	Pos	Pos
Calf	Pos	neg	Yes	Calf	Pos	Neg
Calf	Pos	neg	Yes	Calf	Pos	Neg
resorb/abortion?	Pos	Neg	No	n/a	Pos	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
DEAD		DEAD-No isolation on Retro or Mammary				
Calf	Neg	neg	Yes	Abortion	Neg	Neg
n/a	Neg	neg	Yes	Calf	Neg	Neg
Calf/Dead	Neg	neg	Yes	Calf	Neg	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a	Pos	neg	No	n/a	Sus	Neg
Calf	Neg	neg	Yes	Calf	Neg	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Neg	neg	No	n/a	Neg	Neg
n/a	Pos	neg	No	n/a	Neg	Neg
n/a	Pos	neg	Yes	Calf	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
DEAD		DEAD	B. abortus BV1; retro LN at death			
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Pos	neg	No	n/a	Pos	Neg
n/a	Neg	neg	No	n/a	Neg	Neg
			Yes	Calf	Neg	Neg
			Yes	Stillbirth/huge calf	Neg	Neg

[illegible]

Pregnant 2016	Birth	Serology	Culture
Yes?	No	Pos	
Yes	Dead calf/breach Euthan. 6/8/16	Pos	Neg
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
Yes	Calf	Pos	Pos
Yes	Calf	Neg	Neg
Yes	Calf	Pos	Neg
Yes	Calf-early/weak	Pos	Pos
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	
No	Shipped to CO		
Yes	Calf	Pos	Neg
Yes?	No	Pos	
Yes	Calf	Pos	Pos
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
No			
Yes	Calf	Pos	
Yes	Calf	Neg	Neg
Yes	Stillborn	Neg	Neg
Yes	Calf	Neg	Neg
Yes	Abortion	Neg	Neg
Yes	Calf	Neg	
No	n/a	Pos	
Yes	Calf	Neg	
no	n/a	Pos	
no	n/a	Neg	
no	n/a	Neg	
no	n/a	Sus	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Pos	
no	n/a	Neg	
Yes	Calf	Neg	Neg
Yes	Calf	Neg	Neg

Pregnant 2016	Birth	Serology	Culture
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
No	n/a	Pos	
Yes	Calf	Pos	Neg
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Abortion	Pos	Pos
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Pos	
No	n/a	Pos	
No	n/a	Pos	
No	n/a	Pos	
Yes	Calf	Neg	Neg
Yes	Abortion	Pos	Pos
Yes	Stillbirth/breach	Neg	Neg
Yes	Calf	Neg	Neg
Yes	Calf	Neg	
Yes	Calf	Neg	
Yes	Calf	Pos	Pos
Yes	Abortion	Pos	Pos
Yes	Calf	Pos	Neg
Yes	Calf	Pos	Neg
No	n/a		
Yes	Calf	Pos	
No	n/a		
No	n/a		
No	n/a		
No	n/a		
No	n/a		
No	n/a		
Yes	Calf	Pos	
Yes	Calf	Pos	Neg
No	n/a		

From: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
To: [Rhyen, Jack C \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#)
Subject: stats again...
Date: Wednesday, May 18, 2011 1:06:46 PM

Okay, I'm confused once more. Could you walk me through this one? I thought we were expecting 100% GonaCon efficacy at least in year one so I am not sure how we get a 5-10% abortion rate in treated animals.

(Embedded image moved to file: pic25936.jpg)

From: [Rhyon, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: strategic and work plans -see what you think
Date: Wednesday, October 29, 2014 10:20:45 AM
Attachments: [WILDIT Work Plan FY2015.docx](#)
[WILDIT Strategic PlanFY2015.docx](#)

WiLDIT Work Plan FY2015

Feral Swine Work:

Continue pig TB and *Brucella* vaccine work. Specifically, obtain Hawaii pigs and start colony. Conduct Texas pig trial with killed M.bovis. Plan for next *Brucella* trial.

Collect VOCs from pigs in experimental infections and any field opportunities that arise.

Conduct oral fluid test to see which bait is most attractive. Collect oral fluids from pigs as opportunities arise.

Begin work on FMD, CSF, and/or ASF.

Elk Work

Repeat *Brucella* natural exposure model experiment.

Continue work on spray-dried vaccine for *Brucella*

Explore surveillance work on or around Hardware ranch

Bison Work

Continue GonaCon studies in MT and at Sanddunes

Compile data for John Eisemann to pursue getting bison on label

Continue Drydart development – small bison and elk study

Pursue GTNP and/or YNP brucellosis vaccination field trial

Continue to assist CSU on assisted reproductive techniques work

Other

Assist Torsten with lipid ELISA development

Polar bear work

Fish brucellosis

WiLDIT Strategic Plan

October 28, 2014

The mission of WiLDIT is to develop science-based solutions to disease problems at the wildlife/domestic animal interface.

To accomplish this mission, WiLDIT engages in 4 separate areas of activity or “strategies.”

1. Consultation – provide information and advice to USDA/APHIS and other State and Federal agencies and research partners on interface disease issues; serve as a liaison with agencies, universities and NGOs.
2. Developmental work – Coordinate and/or conduct developmental work to address VS-specific interface disease problem areas, i.e. vaccines and delivery systems for use in wildlife; remote screening and diagnostic techniques for use in wild populations; and strategies to detect, manage, and eradicate diseases in wild populations. Developmental work is usually accomplished through collaborations with other agencies and universities.
3. Training – Serve as a training resource for the agency concerning interface diseases.
4. Monitoring/Surveillance – Conduct surveillance of wild populations around disease outbreaks or on a continuing basis in endemic areas when requested.

From: [Rebecca K Frey](#)
To: [Jack C Rhyan](#); [Brian J McCluskey](#); [Pauline Nol](#); [Kammy R Johnson](#)
Subject: study proposal for GTNP
Date: Thursday, January 06, 2011 2:03:00 PM
Attachments: [BisonBullShed_phase 2_Dec28.docx](#)
[NPSrequirements.doc](#)

Hi all,

I am attaching what I have done so far on the proposal for GTNP. Kammy.....we really need your re-figured confidence tables and to know if 50 is going to be an adequate sample size given the smaller population. Please everyone put your titles in as they should be....I just made them up as I went if I didn't know your actual title.

I am also attaching the NPS guidelines for proposals. I would suggest you briefly review this document and see if there is any glaring omission in what we are submitting. I thought it was a short enough proposal we didn't need a table of contents.....see if you concur.

Still waiting to hear from FWP if we can continue to use the IACUC committee that approved the 1st phase for this or if we need a new plan.....

I am working with John Henningsen on the description of the area we are going to work in, so that will be better described soon.

Thanks,
Becky

(See attached file: NPSrequirements.doc)(See attached file: BisonBullShed_phase 2_Dec28.docx)

Rebecca Frey, Wildlife Biologist/Disease Specialist
USDA APHIS VS
Bozeman, Montana
(406) 333-4425

(b) (6) cell

Evaluation of bison semen from Grand Teton National Park bulls for *Brucella abortus* shedding

January 2011

Investigators and project personnel:

Jack C. Rhyan ¹ Veterinary Epidemiologist	Rebecca Frey ² Wildlife Biologist/Wildlife Disease Specialist
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Matt McCollum ¹ Wildlife Biologist	Ryan Clarke ⁴ Brucellosis Epidemiologist
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Pauline Nol ¹ Wildlife Veterinarian	Chistine Quance ⁵ Brucellosis Lab Specialist
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Kammy Johnson³
Montana Area Epidemiologist

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Abstract:

As brucellosis in domestic cattle has been eradicated across the United States, the wildlife reservoir in the Yellowstone and Grand Teton National Park areas has become the focus of mitigation and prevalence reduction activities. Venereal transmission of brucellosis is examined here in order to determine the importance of treating bull bison in any prevalence reduction program. This study focuses on detection and quantification of brucellosis in the semen in order to determine if venereal transmission could occur. It requires capture of bull bison and collection of semen for *Brucella abortus* culture as well as investigation of reproductive tract lesions that are indicative of brucellosis infection. Results of this study will be useful in determining long term strategies for brucellosis management in bison.

Key Words: Brucellosis, Bison, Venereal Transmission

Background:

Yellowstone and Grand Teton bison have been infected with *Brucella abortus*, the causative agent of bovine brucellosis, since at least 1917. The disease likely originated from exposure to infected cattle. Approximately 50% of these bison have antibodies to *B. abortus*, indicating exposure to the agent and, when rigorously cultured for the organism, over 40% of the seropositive animals are culture-positive (Cheville et al., 1998; Roffe et al., 1999). Studies have shown the disease causes abortion and reproductive tract lesions in bison (Rhyan et al., 1997; Rhyan et al., 2001).

In cattle, venereal exposure is not thought to be a significant route of transmission of brucellosis. In the only study to investigate venereal transmission in bison (Robison et al., 1998), four calves were born to cows bred by a bull shedding *B. abortus* in the semen. Cows and calves remained seronegative. In another study, bison cows intravaginally inoculated with *B. abortus* strain 19, did seroconvert but were culture negative at necropsy 6 months later (Uhrig and Rhyan, unpublished data). It is known that bison bulls shed *Brucella* in the semen (Robison et al., 1998; Olsen et al., 1999); however, the number of organisms shed per ejaculate is unknown. If few brucellae are shed, then there is little cause for concern, but if numbers approaching an infective dose are shed then further work should be done to examine the possibility of venereal transmission.

The question of venereal transmission in bison should be addressed for the following reasons. Proposals to eliminate brucellosis from the Greater Yellowstone Ecosystem (GYE) bison using nonlethal strategies require testing and treatment of cows to eliminate shedding of the organism at calving (Miller, et al., 2004). If venereal transmission from bulls to cows occurs, even in a minority of breedings, then strategies relying only on preventing shedding by cows would be ineffective. Secondly, strategies to remotely vaccinate bison, especially if extended to adult vaccination, could be tailored to include or not include bulls depending on results of venereal transmission studies. Results of this study would also be useful in determining the risk that infected bulls might pose to cattle in proximity to bison as well.

Pilot work:

Bison bulls in Fort Collins, CO have been repeatedly immobilized with A3080 and electroejaculated for semen evaluation. The procedure is effective and 3 to 7 mls of semen are usually obtained from each bull at each sampling. The entire procedure usually requires less than 10 minutes and bulls recover without incident following administration of the narcotic and xylazine antagonists.

During April and early May of 2010, 39 mature bison bulls were immobilized using A3080 and xylazine, electro-ejaculated and recovered. Semen samples collected by electro-ejaculation were analyzed in the field for semen quality and frozen for microbiologic culture for *B. abortus*. Blood samples were collected from the bulls for serologic examination for antibodies to *B. abortus* and for culture. Cultures were done at NVSL in Ames, Iowa and colony counts of *B. abortus* in blood and semen were reported. Semen quality testing was conducted in the field and included evaluation of concentration, viability, spermatid deformities, and cellular content (leukocytes, etc). Animals were sampled from various locations in Gallatin and Park counties as available on state and federal lands in an effort to obtain samples representative of bison from more than one area. All captured bison were marked with an eartag and an RFID device as well as a non-permanent visual marker to identify bulls already sampled.

Hypotheses:

1. GYE bison bulls do not shed infective doses of *B. abortus* in semen.
2. Semen quality is not affected by *B. abortus* infection in GYE bison bulls.

Study Design and Protocol :

We propose to work will occur in July in Grand Teton National Park and surrounding Federal lands beginning in 2011. We intend to examine the load of *B. abortus* in semen and the effect of infection on semen quality just prior to and during breeding. A comparison of the initial study conducted in 2010 in Montana with this study will look for differences in infection and semen quality by time and area.

Similar procedures will be used for the GTNP study as were used in Montana with modifications for the proposed study area. Specifically, we intend to limit our study area to places that bison frequent in the area between the Teton Park Road and the Snake River north of the seasonal road to the Bar BC Ranch to the area known as the potholes. This area is likely to be less traveled by visitors and provides adequate space to find bull bison that will be suitable candidates for the study providing safety for both visitors and bison.

The purpose of capturing bulls in this study is to perform breeding soundness examinations and collect semen and blood samples from individual animals. Semen samples will be evaluated microscopically for semen quality using breeding soundness criteria and cultured for *B. abortus*. Serum will be tested for *B. abortus* antibodies using standard approved laboratory tests.

Bulls will be selected that are 2 years of age or older, with a final goal of 75% of bulls being over 3 yrs of age. Aging will initially be done via visual inspection and expertise by personnel and confirmed through annual incisor eruption. A two year old bull will be identified as having 1 permanent incisor, 3 yr. old with 2, 4 yr. old with 3, and 5 yr. old or older bulls with 4 permanent incisors.

Immobilization Drugs/Dosage: A3080, .01-.015 mg/kg, IM-dart; from the ground
Reversed with Naltrexone, 0.3-0.4 mg/kg. ½ Intramuscular (IM),
½ Sub-cutaneous (SQ)
Xylazine, .07 mg/kg, IM-dart; from the ground
Reversed with Tolazoline, 0.4-0.7 mg/kg. ½ Intravenous (IV), ½
IM

For possible human exposures, syringes with appropriate levels of Naloxone will be on hand and a dose drawn up and prior to A3080 use.

Capture Protocol:

Bison groups in the area will be evaluated for suitability of capture and appropriate resources will be prepared for a capture operation (load darts, have packs and sample collection materials ready etc.). Bison will be spotted and bulls evaluated for appropriate age for the study. When an animal is selected, the capture team will approach the animals to within 50 meters or (preferably) less. When the bison gives the shooter an appropriate shot, a dart will be administered. Once it is confirmed that the dart penetrated the animal, one person will monitor the time. The animal will be closely observed until it is recumbent and immobile. A second dart with a full dose will be ready in case it is needed (if animal is not immobilized after 20 minutes). If a bison is down, but not fully immobilized, the option of a hand injection from a syringe may be considered to induce a deeper plane of anesthesia. Once down, the bison will be hobbled, eye ointment applied, and blindfolded to ensure the safety of the crew and bison. A shade will be erected over the animal, and a team member will immediately begin TPR.

Upon completion of the sample collections and marking of the downed bison, all unnecessary materials and personnel will be removed from the area and the bison will be evaluated for wounds or injury. The person in charge of the capture will administer the reversal agents. Once reversed, personnel will pull back to a safe distance to monitor the bison, watch him get up and continue monitoring for up to 30 minutes post reversal.

Sample/Data Collection:

Bison will be aged by tooth eruption by looking at the incisors. Data will be collected on a pre-made form that provides for all of the needs of the project.

Temperature, pulse and respiration will be recorded at 5-7 minute intervals.

An eartag will be placed in the left ear of each animal noting that the animal has been administered narcotics and a phone number to call. A metal USDA tag will be placed in the right ear. RFID may be placed in the right ear of each animal as well. A small patch of hair will be shaved on the hips of each animal to visually identify animals already captured from a distance while reducing overall visual marking of the animals.

Blood samples will be obtained via jugular venipuncture and collected in 2 10 ml clot tubes and 1 7ml heparinized tube for serology and culture.

After ensuring the animal is appropriately immobilized and restrained, a complete breeding soundness examination will be conducted for each bull using the criteria and methods as established and published by the Society for Theriogenology (SFT). The sample collection expert will measure scrotal circumference and examine the testes and epididymides for abnormalities. The accessory sex organs will also be examined by rectal palpation and abnormalities or pathology noted. Semen samples will be obtained using massage or electroejaculation using warmed collection cup or tubes to avoid cold shock to the sperm.

The semen sample will be evaluated microscopically and scored for gross and individual motility using SFT criteria. An eosin-nigrosin stained sample will be prepared for microscopic evaluation of sperm morphology. The stained slides will be quickly examined to ensure they are of adequate quality to conduct a full spermogram at a later time. Once an adequate slide has been prepared, the remaining semen sample will be preserved for culture in WHO media.

The preserved semen sample and the whole blood sample will be submitted to the National Veterinary Services Laboratory in Ames IA for *B. abortus* culture using standard techniques. Any remaining semen or blood samples will be destroyed via autoclave. Blood serum sample will be submitted to the Montana Department of Livestock Veterinary Diagnostic Laboratory for serologic testing using 7 standard tests including florescent polarization (FPA), card, standard plate (SPT), standard tube (STT), buffered acid plate (BAPA), complement fixation (CF) and rivanol. Any leftover serum will be banked and kept in a -70 freezer until the completion of the study.

General Safety Considerations:

All field personnel will be officially trained or have an understanding of the potential hazards of the immobilization drugs used on the project. All care will be taken to avoid needle sticks, and no unnecessary personnel will be present while narcotics are being handled. Reversal agents and a first aid kit will always be available. The study areas are within 20 miles of professional health care.

The nature of this project provides protection from non-target species as we will be spotting and targeting bull bison for immobilization. The immobilization drug combination is regularly used in large ungulate's and has been found to be quickly metabolized in the target animal leaving very little residue in the tissue soon after animals are recovered with no adverse affects on predators that may come in contact with the target animal.

Darts will be immediately removed from the animal and the injection site marked to avoid contact. All darts that either fall out before animal is down, or do not hit an animal will be searched for and recovered if possible. All darts will be labeled "DANGER, DO NOT TOUCH". All available personnel will be mobilized for a minimum of 15 minutes to search for missing

darts. The recovery of all darts may be difficult due to environmental conditions such as high forage, dense brush or if an animal temporarily evades visual monitoring.

Bison will be captured in locations that are safe for both bison and staff. Unsafe locations may include waterways, roads or other obstacles that interfere with the immobilization of bison. One person will be responsible for monitoring other nearby bison to alert the crew of any dangerous behavior. Solitary bull bison will be the primary subjects in order to avoid behavioral changes in bulls due to the rut.

Grizzly bears frequent the proposed study area and will be active during the project. Field crews will be alert for bears in the area, and will carry bear spray at all times.

As many of these bison may be infected with brucellosis, all personnel handling bison will wear protective gloves, and take precautions to not come in direct contact with infected materials. All crews will carry disinfectant to thoroughly clean/wash if needed.

Euthanasia/Disposal:

If the circumstances indicate that the euthanasia of a bison subject may be warranted, a final evaluation will be made by a veterinarian. The euthanasia procedure will involve the animal being rendered insensible with a captive bolt and then immediately exsanguinated. Under certain circumstances an alternative method using an intravenous injectable euthanasia solution (FATAL-PLUS®) will be employed.

The carcass will be removed immediately after euthanasia as the study area is grizzly bear habitat. As there will possibly be visitors in the same general area as this study it will be imperative to remove attractants for bears causing conflicts for both visitors and the study team. Two staff members will remain with any euthanized animal until it can be removed in order to deter consumption by any other species, particularly if euthanasia solution is indicated. Horses, All Terrain Vehicles or humans will move the carcass to a point where it can be loaded into a vehicle. The vehicle will transport the carcass to the local bear-proof refuse collection site or as directed by NPS staff.

This activity may call for access to otherwise restricted areas, and will be evaluated with NPS staff of a case by case basis. However, it is unlikely that disposal of a euthanized bison will be necessary as mortality due to capture operations from ground darting with the proposed pharmaceuticals is very low.

Communications:

Teams will be equipped with at least one cell phone for all communications within and outside of the capture team.

Personnel will be briefed on the days' activities prior to going into the field.

Travel Methods:

Access to the study area will be done by horseback from the nearest parking area/trailhead. Horses will be used to find and follow bull bison after immobilization is reversed. Horses used in the study will meet import requirements for the state of Wyoming and will be on a weed free hay diet prior to arrival and for duration of project. All personnel either use horses as a regular tool for personal or professional purposes or have used horses as part of other research activities and are familiar with safety considerations regarding horses.

Justification of sample size:

Current data suggests that nearly 60% of the bison bulls randomly selected will be seropositive for *B. abortus*; however, the prevalence of shedding the organism in the semen is currently unknown. Previous studies (Robison et al., 1998; Olsen et al., 1999) show only a few bulls will be shedding at detectable levels at any one sampling. Assuming a population of approximately 300 bulls in GTNP and that 20% of seropositive bulls (10% of all bulls) will be

shedding *B. abortus* in the semen, at least 50 bulls will be sampled. Sampling this number of bulls assures, with 99% confidence, of finding at least one bull shedding *B. abortus* in semen during the sampling period. In addition, this sample size provides a robust number of animals to evaluate different levels of semen quality between seropositive and seronegative bison bulls during two different time periods.

It is noteworthy that the population size, the level of confidence desired, and the prevalence of shedding *B. abortus* all affect the number of bulls necessary to sample to assure finding bulls shedding the organism in semen (Tables 1 and 2).

Table 1. Sample size required to find, with 99% confidence, at least one bison bull shedding *B. abortus* in semen for different herd sizes and prevalence of shedding.

Sample size required	87	43	21	13	86	43	20	13
Probability of detecting at least one infected animal for assumed prevalence	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Number of diseased in population	75	150	300	450	50	100	200	300
Population size	1500	1500	1500	1500	1000	1000	1000	1000
Prevalence (%)	5	10	20	30	5	10	20	30

Table 2. Effect of level of confidence of finding at least one shedding bison bull on sample size calculations for

Sample size required	57	28	13	8	57	28	13	8
Probability of detecting at least one infected animal for assumed prevalence	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Number of diseased in population	75	150	300	450	50	100	200	300
Population size	1500	1500	1500	1500	1000	1000	1000	1000
Prevalence (%)	5	10	20	30	5	10	20	30

Table 2 demonstrates that testing 50 bulls each for Phase 1 and Phase 2 will assure finding at least one bull shedding *B. abortus* with a high level of confidence (95%) even if the prevalence of shedding is lower than first assumed.

Timeline: Start Date: July 2011, work until 30days prior to beginning of hunting season

Capture operations will resume the following July and cease 30 days prior to hunting season until the minimum sample size is met. We anticipate that this will take at least 2 seasons and possibly three with an expected end date of Aug. 1 2013.

An annual summary of capture and preliminary test results will be reported after each capture season. After the sample size is met, final analysis, reporting and publication will occur. We expect to publish results of the study in the Journal of Wildlife Diseases.

Future work:

If results from this study show that some bulls shed large quantities of brucellae in an ejaculate, then a definitive study utilizing natural mating between infected bulls and unexposed cows will be conducted in the future at a remote facility. Alternatively, if no shedding or minimal shedding is detected, the first hypothesis will be considered correct.

References:

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Qualifications of Personnel:**Rebecca Frey:**

Safe-Capture International Wildlife Immobilization Certification; September 2010

Wildlife Immobilization Certification; MTFWP 2000

Regularly chemically immobilize bison with narcotics in current position as Wildlife Disease Biologist at Bison Quarantine Feasibility Study

Jack Rhyan, DVM:

USDA/NRA Firearms Training Course, Fort Collins, CO 2008

In current position, regularly immobilize and recover bison with narcotics.

Ryan Clarke, DVM:

Safe-Capture International Wildlife Immobilization Certification; September 2010

Regularly immobilize bison during research activities associated with current position.

Matt McCollum:

Chemical Immobilization of Wildlife course; Dr. Terry Kreeger; sponsored by Wildlife Pharmaceuticals 2002

Regularly chemically immobilize bison with narcotics in current position as wildlife biologist.

Pauline Nol, DVM:

Regularly immobilize wildlife in current position at Fort Collins research station.

Bison immobilization workshop; Todd Schury/Nigel Caulkett; Parks Canada/University of Saskatchewan 2008.

Kammy Johnson, DVM:

Conducted breeding soundness examinations (BSEs) on bison at Custer State Park during doctoral work. Specialized in bull reproductive work during private practice and was the veterinarian responsible for the health and evaluation (including BSEs) of a bull test station (population 125-175 bulls). Doctoral work involved the BSEs for over 2000 bulls (title: An Observational Study of Breeding Soundness Examinations of Beef Bulls).

GUIDELINES TO RESEARCHERS FOR STUDY PROPOSALS



United States Department of the Interior National Park Service

Your proposal should include each of the required information items listed below, in enough detail that an educated non-specialist can understand exactly what you plan to do. If you have already prepared a relevant proposal for a funding application, work plan, formal agreement, or similar document, then your original proposal likely will satisfy National Park Service (NPS) proposal requirements. The primary area where new information may be necessary concerns the ability of the park to assess what, if any, impacts your research may have on park resources. You should compare your original proposal to these guidelines to be certain that you have provided all the required information. If additional information is required, you can provide it in a cover letter or supplement to your proposal, as appropriate. If a required topic does not apply to your proposed study, simply list the topic and write “not applicable.”

The length of your proposal depends primarily on the complexity of the work planned. In some cases, a proposal may consist of a couple of pages for a study expected to have no significant impact on park resources or visitor experiences. However, proposals for lengthy or complex research problems, for extensive collecting, and for work with special status species or sensitive cultural resources are typically longer, more detailed, and well-organized. Incomplete, disorganized, or illegible proposals may be returned for revision.

I. INTRODUCTION

- A. **Title**
- B. **Date of proposal**
- C. **Investigators** - Provide the name, title, address, telephone number, FAX number, email address, and institutional affiliation of the principal investigator and the name and affiliation of all additional investigators listed in the proposal.
- D. **Table of contents** - Recommended for long or complicated proposals.
- E. **Abstract** - Provide a brief summary description of the proposed project. Include up to five keywords that can be used by the NPS to quickly identify the proposal subject (for example, microbiology, geology, ecology).

II. OVERVIEW - Summarize the proposed project by describing in general the problem or issue being investigated as well as any previous pertinent research.

- A. **Statement of issue** - Describe the issue to be investigated and its importance and relevance to science and to the park. Provide relevant background information that clarifies the need for the project and why it is valuable for the research and/or collecting to be conducted in the park.
 - B. **Literature summary** - Summarize the relevant literature regarding the issue, problem, or questions that will be investigated.
 - C. **Scope of study** - Describe the overall geographic and scientific scope of the project.
 - D. **Intended use of results** - Describe how the products will be used, including any anticipated commercial use.
- III. **OBJECTIVES/HYPOTHESES TO BE TESTED** - Describe the specific objectives of the proposed project. Where appropriate, the objectives should be stated as specific hypotheses to be tested.
- IV. **METHODS** - Describe how the proposed methods and analytical techniques will achieve the study objectives or test the stated hypothesis/question. Provide pertinent literature citations.
- A. **Description of study area** – Clearly describe the study area in terms of park name(s), geographic location(s), and place names. Provide maps, park names, or geographic coordinates as appropriate. Indicate whether your work will take place in an area designated or managed as “wilderness” by the NPS.
 - B. **Procedures** - Describe the proposed study design that addresses the stated objectives and hypotheses. Explain the methods and protocols to be employed in the field and laboratory.
 - C. **Collections** - Describe the type, size, and quantity of specimens or materials to be collected, sampled, or captured, and your plans to remove them from the collecting site. If you are aware specimens of the proposed types already exist in a repository, explain why additional collecting is necessary. Provide scientific nomenclature where possible. Provide information on all other applicable federal or state permits where required.
 - D. **Analysis** - Explain how the data from the study will be analyzed to meet the stated objectives or test the hypotheses. Include any statistical techniques or mathematical models necessary to the understanding of the analysis.
 - E. **Schedule** - Provide a schedule that includes start of project, approximate dates or seasons of fieldwork, analysis, reporting, and completion dates.

- F. **Budget** - Briefly outline the expenses associated with this project and identify your expected funding source(s). Include the anticipated costs pertaining to the cataloging of collected and permanently retained specimens or materials.

V. **PRODUCTS**

- A. **Publications and reports** - Describe the expected publications or reports that will be generated as part of this study.
- B. **Collections** – Describe the proposed disposition of collected specimens or materials. If you propose that the NPS lend the specimens or samples to a non-NPS institution for long-term storage, identify that institution and give a brief justification for this proposal.
- C. **Data and other materials** - Describe any other products to be generated as part of the project, such as, photographs, maps, models, handouts, exhibits, software presentations, raw data, GIS coverages, or videos, and the proposed disposition of these materials. If data are to be collected from the public as part of this study, provide a copy of the data collection instrument (survey, questionnaire, interview protocol, etc.).

VI. **LITERATURE CITED** - Include full bibliographic citations for all reports and publications referenced in the proposal.

VII. **QUALIFICATIONS** - Provide a background summary or curriculum vitae for the principal investigator and other investigators listed in the proposal. Identify their training and qualifications relevant to the proposed project and their ability to conduct field activities in the environment of the proposed study area. Describe previous research and collecting in NPS areas, including study and permit numbers if available.

VIII. **SUPPORTING DOCUMENTATION AND SPECIAL CONCERNS** - Provide information on the following topics where applicable. Attach copies of any supporting documentation that will facilitate processing of your application, such as other required federal and state permits, copies of peer reviews, letters of support and funding commitments, and certifications. Collection of information from the public when federal funds are used may require approval from the Office of Management and Budget (OMB). Upon your request, the NPS Social Science Program will advise you on steps needed to obtain this OMB approval.

- A. **Safety** - Describe any known potentially hazardous activities, such as electrofishing, rock climbing, scuba diving, whitewater boating, aircraft use,

wilderness travel, wildlife capture, handling or immobilization, use of explosives, etc.

- B. **Access to study sites** - Describe the proposed method and frequency of travel to and within the study site(s). Explain any need to enter restricted areas. Describe duration, location, and number of participants for planned backcountry camping.
- C. **Use of mechanized and other equipment** - Describe any field equipment, markers, or supply caches by type, number, and location. You should explain how long they are to be left in the field. Explain the need to use these materials in restricted areas and the alternatives that were considered.
- D. **Chemical use** - Identify any chemicals and hazardous material that you propose using within the park. Indicate the purpose, method of application, and amount to be used. Describe plans for storage, transfer, and disposal of these materials and describe steps to remediate accidental releases into the environment. Attach copies of Material Safety Data Sheets.
- E. **Ground disturbance** - Describe the type, location, area, depth, number, and distribution of expected ground-disturbing activities, such as soil pits, cores, stakes, or latrines. Describe plans for site restoration of significantly affected areas.

Proposals that entail ground disturbance may require an archeological survey and special clearance prior to approval of the study. You can help reduce the extra time that may be required to process such a proposal by including identification of each ground disturbance area on a USGS 7.5-minute topographic map.

- F. **Animal welfare** - For vertebrate species that require review by your Institutional Animal Care and Use Committee (IACUC) according to the Animal Welfare Act, please include a photocopy of the study protocol, and IACUC review form and approval.

For vertebrate species not requiring IACUC review, describe your protocol for any capture, holding, marking, tagging, tissue sampling, or other handling of these animals (including the training and qualifications of personnel relevant to animal handling and care). Please discuss alternative techniques considered and outline any procedures to alleviate pain or distress. Include contingency plans to be implemented in the event of accidental injury to or death of the animal.

- G. **NPS assistance** - Describe any NPS field assistance you would like to receive to complete the proposed study, such as use of equipment or facilities or assistance from staff.
- H. **Wilderness “minimum requirement” protocols** - If some or all of your activities will be conducted within a location administered by the NPS as a

designated, proposed, or potential wilderness area, your proposal should describe how the project adheres to wilderness “minimum requirement” and “minimum tool” concepts. Refer to the park’s wilderness management plan for further information.

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: test charts chute side
Date: Wednesday, March 26, 2014 9:40:12 AM
Attachments: [Feb 26 tests.pdf](#)
[Feb 25 tests.pdf](#)
[March 4 tests.pdf](#)
[March 5 tests.pdf](#)

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Nol, Pauline - APHIS
Sent: Monday, March 24, 2014 2:04 PM
To: Frey, Rebecca K - APHIS
Subject: RE: conference call

Hey Becky,

Could you send the final numbers for how many new bison we got for Gonacon? So I can pretend I'm prepared for tomorrow's meeting;)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Monday, March 24, 2014 10:41 AM
To: Nol, Pauline - APHIS
Cc: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS
Subject: Re: conference call

Oh I suppose. :-). 2 on Tuesday.... That's tomorrow.

Becky
USDA APHIS VS
Sent from my iPhone

On Mar 24, 2014, at 9:59 AM, "Nol, Pauline - APHIS" <Pauline.Nol@aphis.usda.gov> wrote:

Can we move to 2pm?
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126

Cell: (b) (6)
Fax: 970-266-6157

From: Frey, Rebecca K - APHIS
Sent: Monday, March 24, 2014 9:14 AM
To: Rhyan, Jack C - APHIS
Cc: Nol, Pauline - APHIS; Clarke, Patrick R. - APHIS; McCollum, Matthew P - APHIS
Subject: Re: conference call
Tuesday at 1:00. I will send reminder with call in number.

Becky
USDA APHIS VS
Sent from my iPhone

On Mar 21, 2014, at 2:51 PM, "Rhyan, Jack C - APHIS"
<Jack.C.Rhyan@aphis.usda.gov> wrote:

Monday or Tuesday work for me at any of the times. Wednesday is out.
Thanks,
Jack

From: Nol, Pauline - APHIS
Sent: Friday, March 21, 2014 11:35 AM
To: Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS
Subject: RE: conference call

I'm pretty open those days too.

I think Molly just wants to talk with folks and get a feel for what ya'll do. If there is something to participate in then wonderful but otherwise no stress.

I do have a vet student coming for an externship in about two weeks who could theoretically come up for a week and help with potential calvings if possible. Maybe he could ride with Brent a bit too, he he.

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-STAS
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: (b) (6)
Fax: 970-266-6157

From: Clarke, Patrick R. - APHIS
Sent: Friday, March 21, 2014 9:27 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS
Subject: RE: conference call

Any of those dates and times good for me

P. Ryan Clarke, DVM, MPH
Regional Epidemiologist-GYA
USDA-APHIS-VS-WR
406-388-5162

From: Frey, Rebecca K - APHIS

Sent: Friday, March 21, 2014 9:12 AM

To: Clarke, Patrick R. - APHIS; Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: conference call

Can we schedule a call for next week, Tuesday or Wednesday, to discuss the GonaCon project moving forward? Pauline, maybe we can talk about the student request too and figure something out.

I will throw out 1, 2 or 3 pm either day as a starting point.

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

Feb 26, 2014 NEG CUT = 87.8

Date

Page 1 of 5

Trap side recorder Day, Jeremy

Time blood draws begin
Time blood draws end
Time test results available

PA Result	Card Test Result	Serum tube # (SNV) Test Result	Disposition H = hold C = c-section V = vaccinate	Back tag #	Ear tag #	Sex M/F	Age Horn (A,Y,C) Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
264.4			JOHN	8105	YNP 110	F	A 2P		SNIFF
246.4			JOHN	6688	930 279	F	A 4P		NO SNIFF
82.1			C	6689		F	A 2P		SNIFF
194.8			JOHN	6690	930 325	F	A 4P		just erupted SNIFF
226.6			JOHN	6691		F	A 2P		
241.0	+		H	6692	229	M	Y OP		110228
83.4			C	6693		F	C OP		
81.0	+		C	6694		M	C OP		
75.9			C	6695		F	C OP		
158.8	+		C	6696		M	C OP		
88.6			C	6697	930 215	F	A 4P		At 20 mL = 95.2
207.3			JOHN	6698	930 103	F	A 4P		SNIFF
221.9			JOHN	6700	930 141	F	A 4P		SNIFF
198.1			JOHN	6701	930 448	F	A 4P		
83.6	-		H	6702	229	M	Y OP		110229

004307

NEG CUT = 87.8

Page 2 of 5

Date Feb 26 2014

Time blood draws begin
Time blood draws end
Time test results available

004308

rap side recorder Das, Gervina

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag # YNB	Sex M/F	Age Horn (A,Y,C)	Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
102.7	+		H	6703	231	M	2Y	1P		110233
112.1			C	6704		F	A	4P		
85.9			C	6705		F	C	O		
235.4			JOHN	6706	930 462	F	A	4P		SNIFF
87.1			C	6707	930 415	F	A	2P		SNIFF
84.2	-		C	6708		M	C	OP		
85.8			C	6709		F	C	OP		
261.8			JOHN	6710	930 157	F	A	2P		SNIFF
76.9	-		APHIS	6711		M	C	OP		
86.1			C	6712		F	C	OP		
84.3			C	6713		F	A	4P		
88.4	±		H	6714	232	M	Y	OR		110232 A+20.L= 89.2
141.0			JOHN	6715		F	2Y	1P		Pregnant / SNIFF
85.0	-		H	6716	233	M	Y	OP		110233
139.6			C	6717	930 452	F	A	4P		

Feb 26 2014 NEG COT = 87.8

Page 3 of 5

Time blood draws begin
Time blood draws end
Time test results available

004309
Trap side recorder Dog, Geremia

PA Result	Card Test Result	Serum tube # (SN ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
249.2			JOHN	6718	930 294	F	A	4P		SNIFF
190.7			JOHN	6719	930 203	F	A	2P		SNIFF
75.7	+		C	6720		M	C	OP		
104.5			C	*6721	930 564	F	A	4P		
77.8			C	6722		F	A	4P		
94.0	+		APHIS	6723	234	F	Y	OP		A+ 20 uL 165.1
74.0	-		H	6724	235	F	Y	OP		
153.0	±		H	6725	236	M	Y	OP		
106.8	-		H	6726	237	M	Y	OP		
74.4			C	6727		F	C	OP		
81.4			C	6728		F	C	OP		
107.2			C	6729	930 184	F	A	4P		
229.2			JOHN	6730		F	A	4P		
127.7			C	6731		F	A	4P		
101.6			JOHN	6732	Y059	F	A	4P		LOTEK

NEG CUT = 87.8

Date Feb 26 2014

Page 41 of 5

0043

Trap side recorder Daisy Green

Time blood draws begin
Time blood draws end
Time test results available

TPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consup V=vaccinate	Back tag #	Ear tag #	Sex	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
80.9			C	6733		F	A	4P		
74.8			C	6734	930 118	F	A	4P		
104.0			C	6735	930 345	F	A	4P		
223.6			JOHN	6736	930 612	F	A	4P		
77.4	-		ADPTS	6737		M	C	O		
125.6			C	6738	930 275	F	A	4P		SNCRF
67.6			C	6739		F	C	OP		
177.9			JOHN	6740	930 586	F	A	2P		SNCRF
84.4	-		C	6741		M	C	OP		
82.3			C	6742	930 614	F	A	4P		
124.4			C	6743		F	A	4P		
79.7			C	6744		F	A	4P		
79.4	-		C	6745		M	C	OP		
183.8			JOHN	6746	YELL- 400	F	A	4P		
80.9	+		C	6747		M	C	OP		

NEG CUT 87.8

Date Feb 26, 2014

Page 5 of 5

Trap side recorder Doug, Cerezo

Time blood draws begin
Time blood draws end
Time test results available

PPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vacenate	Back tag #	Ear tag #	Sex M/F	Age Horn (A,Y,C) Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
72.2			C	6748		F	C OP		
172.9	+		H	6749	238	M	A 2P		
74.3			C	6750		M	C OP		
175.2	+		ARHS	6751	239	F	Y OP		110239
				6752					
				6753					
				6754					
				6755					
				6756					
				6757					
				6758					
				6759					
				6760					
				6761					
				6762					

Neg Cut off 104.8

Trap side recorder DWS, Cetera

Time blood draws begin

Page _____ of _____

004312

1-406-848-7293

Corral Operations

Feb 25 14:05:37p

trap side recorder

DUG CLEVERIA

Time blood draws begin
Time test results available

Page 5 of 5

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H = hold C = consign V = vaccinate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
102.2	-	-	C	6624	YNP130	F	A	ZP		Sniffer
* 97.3	-	-	C	6625		F	A	ZP		Sniffer
* 107.8	+/-	+	C	6626	779	M	Y	O		106.3 @ 20
108.6	-	-	APHIS	6627		F	Y	O		13.4 @ 20 Neg
144.8	+/-	+	C	6628	780	M	Y	O		
166.4	-	-	APHIS	6629	781	M	Y	O		
172.7	+	+	C	6630	782	M	Y	O		
271.7	+	+	APHIS	6631	783	F	Y	O		
98.8	-	-	APHIS	6632	784	M	Y	O		Pos
95.4	-	-	APHIS	6633	785	F	Y	O		Neg
160.2	-	-	APHIS	6634	786	M	Y	O		Neg
183.5	+	+	APHIS	6635	787	F	Y	O		Pos
256.0	+	+	John	6636		F	A	ZP		Sniffer
97.4	-	-	APHIS	6637	787	F	Y	O		
99.8	-	-	C	6638		F	C	O		Neg
Calves 14		FA		15 cows						

004312

15 cows
14 calves
10 bulls

Neg Cult off 104.8

Page 2 of 5

Date Feb 25 2011

Time blood draws begin
Time blood draws end
Time test results available

00431

Trap side recorder

BP Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vacinate	Back tag #	Ear tag #	Sex M/F	Age Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
263.2	+/	+	John	6639		F	A	3P		Sniffer
228.5	+/	+	C	6640	788	M	2Y	1P		
254.0	+	+	C	6641	789	M	A	2P		exposed
227.8	+	+	John	6642		F	A	2P		Sniffer
98.1	-	-	C	6643		F	C	OP		
98.3	-	-	C	6644		M	C	OP	*	
95.6	-	-	APHIS	6645	790	F	Y	OP		neg
94.3	-	-	APHIS	6646	791	F	Y	OP		neg
95.9	-	-	APHIS	6647	792	M	Y	OP		
254.6	+/	+	C	6648	793	M	Y	OP		
244.2	+	+	C	6649	497	F	A	4P		
94.3	-	-	C	6650		F	C	O		
92.6	-	-	C	6651	494	F	A	4P		
96.1	-	-	C	6652		F	C	O		
143.6	+/	+	C	6653		F	C	OP		

N1 = 89.6
 N2 = 89.8
 N3 = 84.9
 Date = 151.3

89.8 Neg Cutoff 99.8

Time blood draws begin
 Time blood draws end
 Time test results available

0043

Trap side recorder

TPA Result	Card Test Result	Serum tube # (SN ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag #	Sex M/F	Age Horn (A,Y,C) Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
265.8	+	+	APHIS	6654	794	F	2Y 1P		open pos
127.5	-	+	C	6655		F	2Y 1P		dry Sniffer
126.6	-	+	C	6656		F	A 4P		
94.1	-	-	APHIS	6657	795	F	1Y OP		Neg
108.4	-	+	C	6658	796	M	1Y OP		
92.5	-	-	C	6659		M	C OP*		
90.2	-	-	C	6660		F	2Y 1P		dry Sniffer
92.0	-	-	C	6661		F	C O		
89.5	+	-	C	6662		M	C O		
				6663		M	C O		
171.6	-	+	C	6664		M	1Y OP		
99.4	-	-	APHIS	6665	797	M	1Y OP		
87.9	-	-	APHIS	6666	798	M	1Y OP		
158.2	-	+	C	6667		F	A 4P		
130.7	+	+	C	6668		F	A 4P		*Chole FP

Date Feb 25 2014

99.8

Page 4 of 5

Trap side recorder Doug, Cerem

Time blood draws begin
Time blood draws end
Time test results available

004315

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
88.6	-	-	C	6669		F	3Y	2P	1	Sniffer
221.0	1/2	+	C	6670		F	A	4P		
180.6	1/2	+	John	6671		F	A			
270.3	+	+	C	6672	799	M	2Y	1P		0799
132.1	1/2	+	C	6673		F	3Y	2P		Sniffer
208.9	+	+	APHIS	6674	850	F	Y	OP		pos
226.8	-	+	John	6675		F	A	4P		
144.9	+	+	C	6676	226	M	C	OP		WP110226
244.2	+	+	APHIS	6677	827	F	Y	OP		pos
88.4	-	-	C	6678		M	C	OP *		
85.0	-	-	C	6679		F	C	O		
87.9	-	-	C	6680		F	A	4P		
231.8	+	+	John	6681		F	2Y	1P		Pregnant Sniffer
230.0	+	+	C	6682		F	C	OP		
127.9	-	+	C	6683	228	M	1Y	OP		

NEG CUTOFF = 93.6

Date Tuesday March 4 2014

Time blood draws begin
Time blood draws end
Time test results available

Page 1 of 24

Trap side recorder

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vacuate	Back tag #	Ear tag #	Sex M/F	Age		Quantity Blood Drawn	Notes Photograph, fecal
							Horn (A,Y,C)	Tooth eruption		
81.2		-	C-Roman	6753	YNP11D 930 197	F	A	4P		SNIFFER
180.8		+	JOHN	6754	930 027	F	A	2P		SNIFFER
78.8		-		6755		M	C	OP		
100.9		+	C-Roman	6756		F	A	3P		SNIFFER
277.4		+	JOHN	6757		F	A	2P		SNIFFER
96.8		+/-	C-Roman	6758		F	A	4P		94.0 @ 20.1 L w/ NEG CUTOFF OF 84.6
84.3		-		6759		M	C	OP		
233.6		+	JOHN	6760	41107B	F	A	2P		Pregnant Remove collar
83.2		-		6761		M	C	OP		
241.7		+	JOHN	6762		F	A	1P		Pregnant SNIFFER
83.6		-		6763		F	C	OP		
219.9		+	JOHN	6764		F	A	3P		SNIFFER
245.7		+	C-Roman	6765	240	M	Y	OP		
263.9		+	JOHN	6766		F	A	1P		Pregnant SNIFFER
88.5		-		6767		F	C	OP		

NEG CUTOFF = 93.6

Date 3-4-14

Page 2 of 4

Trap side recorder

Time blood draws begin
Time blood draws end
Time test results available

FPA Result	Card Test Result	Serum tube # (SN ***)	Disposition H = hold C = cull V = vaccinate	Back tag #	Ear tag # (Y/N) ID	Sex M/F	Age Horn (A,Y,C) Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
129.7	+	+	JOHN	6768		F	A 3P		SMIFFER
79.5	-	-		6769		F	C OP		
88.7	-	-	C-Roman	6770	241	M	Y OP		SMIFFER
83.6	-	-		6771		F	C OP		
77.7	-	-	C-Roman	6772		F	A 2P		SMIFFER
193.7	+	+	JOHN	6773		F	A 4P		
84.1	-	-		6774		F	C OP		
255.2	+	+	JOHN	6775		F	A 4P		
81.4	-	-	C-Roman	6776		F	A 4P		
213.2	+	+	C-Roman	6777	242	M	Y OP		
85.6	-	-	HOLD	6778	243	F	Y OP		
127.4	NEG CUT 84.6	+	JOHN	6779		F	A 4P		
118.7	81.6	+	C-Roman	6780	930 382	M	A 3P		
78.0	84.6	-		6781		F	C OP		
154.9	84.6	+	A-Becky	6782	244	F	Y OP		

SMIFFER

*

*

*

Date 3-4-14

Trap side recorder

Time blood draws begin
Time blood draws end
Time test results available

Page 3 of 4

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
215.4		+	C-RAN	6783	YNP110 245	M	Y	OP		
78.9		-	C-RAN	6784	246	M	Y	OP		
171.5	+	+	Q-Becky	6785	247	F	Y	OP		
247.1		+	C-RAN	6786	248	M	Y	OP		
82.4		-		6787		M	C	OP		
124.1		+	C-RAN	6788		F	A	4P		
112.5	+/-	+	Q-Becky	6789		F	C	OP		Broken Horn 127.4 @ 20uL
180.7	+	+	Q-Becky	6790	249	F	2Y	1P		OPEN
131.7		+	C-RAN	6791	250	M	Y	OP		
261.0		+	JOHN	6792		F	A	1P		SMIFER
93.3		+	JOHN	6793	YNP30 253	F	A	4P		100.7 @ 20uL
232.5		+	JOHN	6794	YEL33	F	A	4P		
82.4		-		6795		M	C	OP		SMIFER
77.7		-		6796		M	C	OP		SMIFER
91.7	+	+	JOHN	6797		F	A	4P		96.5 @ 20uL

NEG CUTOFF = 88.0

Date 3-5-14

Time blood draws begin

Time blood draws end

Time test results available

Trap side recorder Geremia Blanton

FPA Result	Card Test Result	Serum tube # (SN ***)	Disposition H=hold C=consign V=vaccinate	Buck tag #	Ear tag #	Sex M/F	Age Horn (A,Y,C) Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
174.9		+	C-shill	81V5	YNP10	F	A 4P		SNIFER OLD
194.3		+	H	6812	255	M	Y OP		
81.3		-	C-Road	6813	256	F	C OP		Sniffer
80.4		-	H	6814	257	F	Y OP		
76.4		-	H	6815	258	F	Y OP		
81.7		-	C-Road	6816	259	F	C OR		Sniffer
84.3		-	C-Road	6817	260	F	C OP		Sniffer
163.4		+	Colorado	6818	261	F	A 4P		
81.1		-	H	6819	262	F	Y OP		
183.5		+	H	6820	263	M	Y OP		
189.8		+	C-shill	6821	264	F	A 3P		
271.3		+	H	6822	265	M	Y OP		
76.7		-	H	6823	266	F	Y OP		
249.4	+	+	Q	6824	267	F	Y OP		
144.4	+	+	Q	6825	268	F	Y OP		

NEG CUT= 88.0

Date

Page 2 of

Time blood draws begin

Time blood draws end

Time test results available

Trap side recorder

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign Y=vaccinate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
195.9	+	+	Q	6826	269	F	2Y	1P		OPEN
235.3	+	+	Q	6827	270	F	Y	OP		
82.4		-	C-Pow	6828	271	M	C	OP		SNIFFER
87.3	+	-	Q	6829	272	M	A	2P		Empty I ₂
97.1	+	+	Q	6830	273	F	Y	OP		109.2 @ 20uL
245.3		+	C-Still	6831	274	M	A	2P		Empty I ₂
81.0		-	C-Pow	6832	275	F	C	OP		Sniffer
79.8		-	C-Pow	6833	276	M	C	OP		SNIFFER
79.7		-	H	6834	277	F	Y	OP		cow pen
230.0		+	C-Still	6835	278	F	2Y	1P		PREGN.
77.0		-	H	6836	279	F	Y	OP		
138.9		+	C-Still	6837	280	F	A	4P		
268.0	+	+	Q	6838	281	F	2X	1P		OPEN
73.4		-	H	6839	282	M	Y	OP		
78.3		-	H	6840	283	F	Y	OP		

NEG-CUT = 88.0

Date

Trap side recorder

Time blood draws begin
Time blood draws end
Time test results available

Page 3 of

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign X=vacillate	Back tag #	Ear tag #	Sex M/F	Horn (A,Y,C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
120.2		+	H	6841	YNP110	M	Y	OP		
164.1	+	+	Q	6842	285	F	Y	OP		
147.9		+	H	6843	286	M	Y	OP		
178.0	+	+	Q	6844	287	F	Y	OP		
79.3		-	Q	6845	288	M	2Y	IP		
140.5	+	+	C-shill	6846	YNP930 195	F	A	4P		Smitten
79.7		-	C-PLOW	6847	289	M	C	OP		Smitten
130.0	+	+	C-shill	6848	290	F	A	4P		
97.3	+/	+	C-PLOW	6849	291	M	C	OP		
92.6	+/	+/	C-shill	6850	YNP930 768	F	A	2P		SMITTER 102.0 @ 20.1L
80.6	-	-	C-shill	6851	YNP930 180	F	A	3P		95.9 @ 20.1L
282.2	+	+	Q(2)	6852	292	M	A	2P		
76.8	-	-	C-shill	6853	930 109	F	A	4P		SMITTER
246.4	+	+	C-shill	6854	293	F	2Y	1P		PREGN
78.5	-	-	C-shill	6855	Yell 365	F	A	4P		

NEG CUT = 88.0

Date NEG CUT = 87.7

Page 41 of

Time blood draws begin

Time blood draws end

Time test results available

Trap side recorder

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vacillate	Back tag #	Ear tag #	Sex M/F	Horn (A, V, C)	Age Tooth eruption	Quantity Blood Drawn	Notes Photograph, fecal
188.0		+	Q (2)	6856	YNP930 156	M	A	3P		
237.6	+	+	Q	6857	294	F	Y	OP		
248.5		+	C-shill	6858	295	F	2Y	1P		NOT FOR BECKY
80.4		-	H	6859	296	M	Y	OP		
70.0		-	H	6860	297	M	C	OP		
74.1		-	C-shill	6861	930 510	F	A	4P		SNIFFER
70.4		-	C-shill	6862	298	F	A	1P		erupting I2
73.0		-	H	6863	299	M	Y	OP		
76.8		-	H	6864	300	M	Y	OP		
224.7		+	H	6865	201	M	C	OP		
113.3		+	H	6866	202	M	Y	OP		
220.9		+	H	6867	203	M	Y	OP		
78.5		-	C-shill	6868	204	F	C	OP		Sniff
75.2		-	C-shill	6869	205	F	A	2P		
77.6		-	H	6870	206	M	C	OP		

Date

Time blood draws begin
Time blood draws end
Time test results available

Page 5 of

Trap side recorder

FPA Result	Card Test Result	Serum tube # (5N ***)	Disposition H=hold C=consign V=vaccinate	Back tag #	Ear tag #	Sex M/F	Age		Quantity Blood Drawn	Notes Photograph, fecal
							Horn (A,Y,C)	Tooth eruption		
67.8	-	-	C-PION	6871	207 YNP30 375	F	C	OP		SNIFFER
165.2		+	C-SH11	6872	930 356	F	A	4P		SNIFFER
242.3		+	C-SH11	6873		F	A	2P		
76.7		-	H	6874	208	M	C	OP		
278.4		+	C-SH11	6875	209	F	A	4P		SNIFFER
115.5		+	C-SH11	6876	YNP30 237	F	A	4P		
				6876						
108.0		+	C-SH11	6877	YNP30 384	F	A	4P		
154.0		+	Colorado	6878		F	A	4P		
224.8		+	C-SH11	6879	YNP30 386	F	A	4P		SNIFFER
74.6		-	H	6880	210	M	C	OP		
				6881						
				6882						
				6883						
				6884						
				6885						

From: [Frey, Rebecca K \(APHIS\)](#)
To: [Clarke, Patrick R. \(APHIS\)](#); [McCollum, Matthew P \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#); [Nol, Pauline \(APHIS\)](#); [\(b\) \(6\) @aol.com"](#)
Cc: [Herriott, Donald E \(APHIS\)](#)
Subject: Testing gonacon bufs
Date: Monday, August 29, 2011 2:05:59 PM

Hi,

Just following up on a date to retest our GonaCon animals, which we originally intended to do end of Sept. Sept.21 or 22, or the following week? If anyone wants to join from Fort Collins we would be happy! We will likely retest then in early November, maybe preg check just to be sure, unless we have more positives, in which case we will need to test again in December. We plan to have positives and negatives separated before any new animals arrive in 2012.

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: The GonoCon study update
Date: Wednesday, February 11, 2015 11:11:30 AM

Hi all,

I was looking through the study plan (gasp!) and it would seem that we said we would keep at least a subset of the calves through their first calf to monitor calves born to seropositive females. Sooooo, I think it is also cool that we have some that were left in the pen, and some that were not. It may be really neat to compare those two outcomes as well. That means, if you have room and such, that you should prioritize to keep any females from the GonaCon study with an R in their tag number, also including 420, through their 1st calf. Also should probably try not to vaccinate or otherwise defile them ;-)

It said "a subset" so we will have 3 more calving seasons to choose calves from, however after the last one, we will be euthanizing all of the cows so will not have an original pen to keep calves experimentally exposed in. Meaning, we may want to make these 1st 3 calving seasons our priority animals. The exception being the treatment pen, which we may end up keeping longer once we get calves on the ground, in order to have some make it 3 years to calving.

So far, there are no female calves born to infected cows in the GC treatment pen and there are only 2 pregnant this year. Another option is to keep a smaller cohort of control animals (maybe all of the offspring kept so far) another year, or throw any calves into the 2nd cohort.....though that screws with the numbers/pen a bit.

Stuff to chew on, especially since we have thought about getting rid of our 1st control pen given our issues at Franks. Ryan and I will try to come up with some strategies to make Franks work better.....but it will take \$\$\$.

That is all ☺

Becky

Rebecca Frey

Wildlife Biologist/Disease Specialist

USDA APHIS VS

Montana

406-333-4425 office/fax

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Cc: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: update of GonaCon Study IACUC
Date: Tuesday, December 10, 2013 9:41:32 AM

Hi Pauline!

It is only about -30 wind chill here, 40 -70 mph gusts, AWESOME! Can't wait for you all to get here!

I am updating the permits, and see that we have an expiration on our IACUC of May 2014. I am sure we will need to update signatures for another 3 yr. period in order to keep our permits active. Can you initiate a new form for signatures on that? I only have the PDF copy.

Thanks!

Becky

Rebecca Frey

Wildlife Disease Specialist

USDA APHIS Veterinary Services

Montana

406-333-4425

From: [Ahola, Sara C - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: update on activities?
Date: Monday, November 14, 2016 4:07:57 PM

Hello Jack and Pauline,

I have to give a presentation on Wednesday to Foreign Animal Disease Diagnosticians in the Colorado area. Would you be able to provide me with one or two slides on your current activities – GonaCon trial, etc. I'll give you credit of course, and don't worry about formatting them – I'll incorporate. This is only if you have time. . . . maybe whatever Pauline used in Pocatello?

~Sara

Sara C. Ahola, DVM, MA-Econ.
Veterinary Medical Officer-Epidemiology
United States Department of Agriculture
APHIS-Veterinary Services: Surveillance, Preparedness & Response Services
Cattle Health Staff
2150 Centre Ave
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Sara.C.Ahola@aphis.usda.gov

From: [Ahola, Sara C - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: update on activities?
Date: Monday, November 14, 2016 4:07:57 PM

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~Sara

Sara C. Ahola, DVM, MA-Econ.
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From: (b) (6)
To: [Nol. Pauline - APHIS](#)
Subject: updated sample lists
Date: Wednesday, January 16, 2013 6:00:33 PM
Attachments: [Sand Dune Bison GnRH study OA-1923.xlsx](#)
[Montana Bison GnRH sample log.xlsx](#)

Hi,

I attached updated logs, with some minor edits I added. I ran everything which I had two samples for. Also I put back all the extra serum I had into your freezer. I will leave the dilutions in our ELISA freezer.

Best,

(b) (6)

--

(b) (6)

DVM Candidate 2015
M.S. Toxicology
B.S. Environmental Health
Colorado State University
(b) (6) [@rams.colostate.edu](mailto:(b) (6)@rams.colostate.edu)

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: WiLDIT Accomplishments
Date: Monday, October 05, 2015 4:01:44 PM
Attachments: [WiLDIT Accomplishments FY 2015.docx](#)

Hey,

Suelee needs WiLDIT accomplishments. She said we can just list them corporately. I gave it a start and would appreciate you all helping out. Matt, I included the bison transfer but feel free to rewrite it as you wish. Pauline, it needs all your feral swine work and VOCs and papers, talks, etc.

Thanks much

Jack

WiLDIT Accomplishments FY 2015

Bison and elk work:

Continued GonaCon studies in southern Colorado and Montana

Assisted CSU in reproductive work

Played key role in preparations for release of YNP genetics bison on public ground to found the Laramie Foothills Conservation Herd (Nov 1 is release date) and arranged to provide bulls with YNP genetics for founder herd at Midewyn Tall Grass Prairie in Illinois. (Relocation occurs mid-October)

Currently conducting challenge study using finely powdered RB51 compounded with montmorilite in mice. If successful, we will try this approach in elk.

Submitted work for patent on DryDart to ARS patent committee. ARS patent committee accepted assignment and submitted application to Patent office. We are continuing to develop DryDart and are starting live animal study to measure immune response to RB51 delivered by DryDart compared to hand vaccination.

Arranged with the states of Colorado and Wyoming to collect up to 20 *Brucella*-positive, pregnant, wild elk cows at feedgrounds in Wyoming in winter 2016, to be transported to Colorado for a natural *Brucella* transmission study.

Conducted study in collaboration with Colorado Parks and Wildlife and Wyoming Game and Fish Commission on efficacy of a Nalbuphine/Azaperone/Medetomidine drug combination in bison.

Feral Swine:

Continue vaccine study investigating efficacy of killed oral *Mycobacterium bovis* (Spanish and Michigan strains) in feral swine of Texas origin.

Participated in feral swine ear tag study to determine feasible ear tag weights in the context of eventual application of satellite ear tags.

Investigation of use of volatile organic compounds in breath and feces of swine for detection of *Mycobacterium tuberculosis* complex infection. Collected breath and fecal VOCs from wild boar in Doñana National Park in Spain, September 2015, to be analyzed by collaborators at Roviri i Virgili University in Tarragona, Spain. Will collect VOCs from feral swine in an experimental *M. bovis* challenge in Fall, 2015, also to be analyzed by Roviri i Virgili University.

Visited Texas A and M facility in Kingsville, TX to explore possibilities in collaborative feral swine work with researchers at that university.

Working with Hawaii Department of Agriculture to receive feral swine from Molokai, HI for future testing of tuberculosis vaccines.

Cattle

Collected breath and fecal samples from Michigan dairy cattle involved in an outbreak of *M. bovis*. Samples were sent to the Technion, Haifa, Israel for volatile organic compound analysis.

Publications and presentations:

Rhyan JC, Tyers D, Zimmer J, Lewandowski K, Hennager S, Young J, Pappert R, Panella A, and Kosoy, O. 2015. Serologic survey of snowshoe hares in the Greater Yellowstone Area for brucellosis, tularemia, and snowshoe hare virus. *J Wildl Dis* 51:769-773.

USAHA Brucellosis Scientific Subcommittee, Research Updates. October 19, 2014.

Presented Research Updates to National Academy of Sciences, Brucellosis Review Panel September 15, 2015

Presented Research Updates to Brucellosis Research Group in Jackson, WY, Sept 24, 2015.

Stahl R.S., Ellis C.K., **P. Nol**, W.R. Waters, M. Palmer, and K.C. VerCauteren. 2015. Fecal volatile organic compound profiles from white-tailed deer (*Odocoileus virginianus*) as indicators of *Mycobacterium bovis* exposure or *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) vaccination. PLoS One 10(6):e0129740.

Fagre, A., K.A. Patyk, **P. Nol**, T. Atwood, K. Hueffer, and C. Duncan. 2015. A review of infectious agents in polar bears (*Ursus maritimus*) and their long-term ecological relevance. Ecohealth. 2015 Mar 20.

Patyk, K.A., C. Duncan, **P. Nol**, C. Sonne, K. Laidre, M. Obbard, Ø Wiig, J. Aars, E. Regehr, L.L. Gustafson, and T. Atwood. Establishing a definition of polar bear (*Ursus maritimus*) health: a guide to research and management activities. 2015. Sci Total Environ. 514:371-8.

Presented update on wild swine tuberculosis on an international scale and participated in a panel discussion. Many Hosts of Mycobacteria VI: Host Specificity and Dynamics of Mycobacterial Disease, March 26-27, 2015, Tulane National Primate Research Center, Covington, Louisiana,

Presented to undergraduate and graduate students on tuberculosis at epidemiology course at Colorado State University

Presented to students and faculty at the Department of Electronics, Electrical and Automatic Engineering at Rovira i Virgili University, Tarragona, Spain on WiLDIT volatile organic compound research.

Students/Externs:

Eight students were accepted as veterinary externs in FY15. These externs were hosted at NWRC by WiLDIT for two to four week blocks. They represented five veterinary schools in the country. One MPH/veterinary student at Colorado State University was co-mentored over the summer by NWRC researchers and WiLDIT researchers. One local high school student interned for WiLDIT over the summer.

WiLDIT has one Saul T. Wilson Scholar from Colorado State University. This student is participating in lyophilized *Brucella* vaccine research in mice.

WiLDIT is supporting and mentoring one MS student at Colorado State University. This student is a DVM and is participating in feral swine tuberculosis vaccine research.

Served on PhD committee for DVM/PhD student studying brucellosis at Colorado State University.

Five undergraduate students from Colorado State University perform animal care and maintenance at the WiLDIT wildlife research facility. These students are supported through a cooperative agreement with the Animal Population Health Institute at Colorado State University.

Other:

Two WiLDIT members participated in HPAI task force in Minnesota, May 3–June 1.

Two WiLDIT members participated in a regulatory veterinary medicine laboratory at Colorado State University.

Produced a material transfer agreement with NEIKER, Spain to use killed *Mycobacterium bovis* vaccine in feral swine.

Established cooperative agreement with the Department of Electronics, Electrical and Automatic Engineering, Rovira i Virgili University, Tarragona, Spain to conduct volatile organic compound analysis on breath and feces to detect tuberculosis in wild boar and feral swine

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to conduct tuberculosis volatile organic compound and molecular research, and support the wildlife research facility.

Established cooperative agreement with the Department of Pathobiological Sciences to conduct tuberculosis vaccine research in feral swine.

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to support a MS student in research of tuberculosis vaccines in feral swine.

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: WILDIT Activities Report Nov 2012
Date: Wednesday, November 14, 2012 1:45:00 PM
Attachments: [WILDITActivitiesUpdateNov2012.docx](#)

Added a few things. Animal numbers need updating. Did I miss something and do the things I added need to be taken out again?

P

Wildlife/Livestock Disease Investigations Team (WILDIT)

USDA/APHIS/VS, Western Region

Current Projects and Activities Update, November 2012

Projects – Field/animal work completed, laboratory work, data analysis, and/or manuscript preparations ongoing

1. Oral elk vaccine studies with recombinant RB51; 2 studies using vaccine from Virginia Tech. Results of first study were promising. Second study results: vaccine had little effect. Collaborators: ARS, CSU, NVSL, Virginia Tech.
2. Transmission of BCG among white-tailed deer and cattle following oral vaccination of deer. Some deer comingled with vaccinates became skin test positive. Cattle sharing facilities and feeders but not in contact with vaccinates remained negative. Study is one of several needed prior to field trial of vaccine in Michigan. Collaborators: ARS, NVSL, CSU.
3. Risk of *Brucella abortus* transmission posed to cattle and bison by bison or elk abortions. Study demonstrated oral contact of cattle and bison with fetuses in environment. Risk of contact was greater in pregnant animals. Collaborators: APHIS-WS
4. BCG tissue persistence in feral swine. Study designed to determine tissue clearance of orally administered BCG vaccine in feral swine. Study needed prior to eventual BCG field trial on Molokai. Collaborators: ARS, NVSL, CSU.

Projects – Ongoing and planned field/animal work

1. Oral feral swine vaccine studies using 2 candidate rough *Brucella suis* vaccines. Studies to determine protection of 2 candidate vaccines in feral swine. Collaborators: ARS, CSU, Virginia Tech.
2. Detection of volatile organic compounds (VOCs) in breath of animals infected with TB and brucellosis. Studies investigate effectiveness of VOC detection in the breath as a screening/diagnostic tool for TB and brucellosis. 2 TB studies showed promising results. Collaborators: APHIS-WS, ARS, CSU, Israel Institute of Technology.
3. GonaCon™ study in bison herd in southern Colorado. Study investigates safety and duration of infertility in bison vaccinated with GonaCon™ as potential tool to prevent *B. abortus* transmission. Collaborators: APHIS-WS, USGS- BRD, TNC.
4. Lipidomics as a antibody-based diagnostic method for brucellosis and bovine tuberculosis. Collaborators: CSU, NVSL
5. Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic. Prospective and retrospective study of prevalence of brucellosis in polar bear and brown bears and investigation of origin of exposure/infection. Collaborators: USGS-BRD, NVSL
6. Venereal transmission of brucellosis in bison studies. Studies investigate transmission of *B. abortus* by venereal route in bison. First study showed seroconversion following intravaginal inoculation. Second study, a breeding trial, is ongoing. A contraceptive would not be effective in preventing shedding if venereal transmission is common in bison.
7. *Brucella abortus* infection/transmission model in elk. Project to start in winter/early spring 2013. A natural infection/transmission model will be tested in *B. abortus*-infected wild elk obtained from the GYA. If successful, this model will be used for subsequent vaccine studies in this species.
8. Molokai feral swine colony. Establish breeding colony of feral swine originating from Molokai-winter/early spring 2013. These swine will be used for the purposes of tuberculosis vaccine studies.

Commented [pn1]: Put this in even though not happening yet, but will soon.

Commented [pn2]: Take or leave as well

Other WiLDIT activities:

Extension/consultation, One Health participation, Consortium for the Advancement of Brucellosis Science (CABS) and USAHA Brucellosis Scientific Advisory Subcommittee members, instructors in CSU and APHIS courses, and host several externs and visiting scientists each year.

Census of Animals in Fort Collins Facility

Bison: 29 (Yellowstone genetics); 31 owned & maintained by CSU for embryo transfer project.

Commented [pn3]: How many are there currently?

Feral swine: 13 (foundation herd for producing pigs for studies)

White-tailed deer: 13 owned & maintained by APHIS-WS for CWD studies at CSU

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: WILDIT Current and Proposed-Budget and Timeline
Date: Wednesday, November 14, 2012 2:33:00 PM
Attachments: [WILDIT Current and Proposed Work 11-8-12.docx](#)

It's a start...

Pauline Nol, DVM, MS, PhD

Wildlife Livestock Disease Investigations Team

USDA-APHIS-VS-Western Region

National Wildlife Research Center

4101 LaPorte Ave.

Fort Collins, CO 80521

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Cell: (b) (6)

Fax: 970-266-6157

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

On going:

- Evaluation of volatile organic compounds and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals **2010-**
- Lipidomics as a diagnostic method for brucellosis and bovine tuberculosis **2011-**
- Development and evaluation of tuberculosis vaccines and vaccine delivery methods for white-tailed deer and feral swine **2005-2017**
- Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic 2012-2015
- Evaluation of brucellosis vaccines in feral swine **2012-**
- Development of nonlethal methods to eliminate *Brucella abortus* from bison and elk in the GYA **2004-**
- Embryo Transfer as disease mitigation strategy in bison **2011-**

Proposed Short Term

- Inactivated *Mycobacterium bovis* vaccine in feral swine **2013-2014**
- *Brucella abortus* infection model in elk **2013-2014**

Proposed Long Term:

- Elk Genome Project **2013-**
- *Brucella* vaccine studies in elk and feral swine **2012**
- Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic **2012-2015**
- Ecology of diseases in feral swine in the United States **2013-**
- FADs **2014-**
- International work **2013-**

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

On going:

Evaluation of volatile organic compounds (VOCs) and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals

Commented [pn1]: CA budget numbers?

Develop instrumentation that could be utilized in the field to detect brucellosis in wild bison, elk, and feral swine. Study underway is entitled "Detection of volatile organic compounds in bison as tools for detection of brucellosis". Collaborators: CSU, Technion, Israel, ARS.

Develop instrumentation that could be utilized in the field to detect bovine tuberculosis in white-tailed deer, elk, feral swine, and cattle. Study underway is entitled "Detection of volatile organic compounds and bacterial nucleic acids in animals as tools for diagnosis of tuberculosis". Collaborators: ARS, APHIS/WS, CSU, Technion, Israel.

Budget:

\$40,000

Binational Agricultural Research and Development Fund Grant was submitted for *M. bovis* and *M. avium paratuberculosis* in cattle for \$284,000 between CSU and the Technion, Israel.

Development and evaluation of tuberculosis vaccines and vaccine delivery methods for white-tailed deer and feral swine

Determination of persistence of BCG in feral swine orally vaccinated with BCG. Animal work is completed. Culture of tissues pending. Collaborators: NVSL, CSU

Budget (Animal feed and maintenance for 9 months)

\$7,000

Development of vaccine delivery systems for administration of oral BCG vaccine to wild white-tailed deer. Collaborators: APHIS/WS, ARS, MIDNR

Budget (Travel)

\$1,500

Lipidomics as a diagnostic method for brucellosis and bovine tuberculosis

Working with CSU scientists to identify lipids uniquely produced by *Brucella* species in order to identify *Brucella*-infected animals as well as distinguish the particular species of *Brucella*. This work will lead to the development of ELISA tests to serologically distinguish between *Brucella* spp. and to differentiate them from *Yersinia enterocolitica* 0:9 infection. Collaborators: CSU, USDA/ARS.

Budget

To be determined

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic

Investigate the seroprevalence of *Brucella* spp. antibodies in grizzly bears and polar bears in Alaska and the Greater Yellowstone Area. Data will be obtained from archived samples and samples to be collected during future field capture events. Collaborators: USGS, State of Alaska, NVSL, CEAH, State of MT

Budget

To be determined

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Evaluation of brucellosis vaccines in feral swine

Two vaccine candidates are currently being evaluated in feral swine and domestic swine for protection against *B. suis* infection. This is a nonpregnant animal study and includes barrows and gilts. One vaccine candidate is a field strain rough *B. suis* discovered in South Carolina feral swine by ARS/NADC researchers. The second vaccine is an engineered rough *B. suis* developed by Va Tech which contains a plasmid that expresses GnRH protein. Collaborators: VA Tech, USDA/ARS.

Budget (Farrowing, maintenance, travel)

\$4,000

Development of nonlethal methods to eliminate *Brucella abortus* from bison and elk in the GYA

Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison. Demonstrate efficacy of GnRH vaccine (GonaCon™) in producing infertility, thereby preventing transmission of brucellosis, in bison and elk. Study initiated in May, 2012 in Corwin Springs, MT is entitled and will continue through 2017. Research partner agency: WS. Other collaborators: Idaho F&G, WY G&F, MTFWP, NPS, APHIS/WS, CSU.

Budget (Travel, Feed and Maintenance, Drugs, Darts, Lease)

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Evaluation of GonaCon™, an immunocontraceptive vaccine, in free-ranging bison: A pilot study. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in free-ranging female bison on property owned by The Nature Conservancy (TNC) and managed by Zapata Partners (Medano-Zapata Ranch). The property is adjacent to Great Sand Dunes National Park in south central Colorado. Study initiated in November 2011. Ten bison cows were vaccinated with GonaCon™ and ten bison serve as controls.

Budget

\$3,500

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Embryo Transfer as disease mitigation strategy in bison

Development of an effective embryo transfer technique in bison in order to produce disease-free offspring derived from bison with various infections such as brucellosis and paratuberculosis. Collaborators: CSU, WCS, NVSL

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Budget (Tissue handling and laboratory)

\$

Commented [pn5]: Cost?

Proposed Short Term

Inactivated *Mycobacterium bovis* vaccine in feral swine

Spanish researchers at IREC have tested an inactivated *M. bovis* strain for efficacy as a vaccine in wild boar against disease caused by *M. bovis* infection. Administered orally, the vaccine is equally as effective as BCG in wild boar. We propose a study to test efficacy of this vaccine in feral swine. A killed vaccine will be much easier to administer in a field situation. The target population in the US for this vaccine would be the *M. bovis*-infected feral swine population on Molokai Island in Hawaii.

Budget (Purchase and shipment of feral piglets from Hawaii, Feed and maintenance of animals (non-BSL3), Animal Care, Animal per diem BSL 3, Laboratory/Immunology, Tissue Culture, Histologic Preparation

\$85,000

***Brucella abortus* infection model in elk**

Brucella abortus is extremely expensive and difficult to work with due to its status as a select agent. In addition, experimental infection models in elk have not been very successful in mimicking natural infection events. We propose to develop a natural infection model for *B. abortus* in elk that would allow test animals to be held in outdoor pens, therefore reducing costs as well as stress on the animals that would otherwise be required to be held in BSL-3 facilities. This study would involve capturing 3 year old pregnant *B. abortus*-infected elk from the wild as well as nonpregnant, 2-3 year old uninfected elk. Infected and uninfected elk will be housed in the same double-fenced paddock and infected elk will be allowed to calve and abort in the pen. The uninfected elk will be thus naturally exposed to infected fetuses, placentas, and/or discharges. These exposed elk will be bred and held for another year to monitor whether they seroconvert and abort the following calving season. If this approach results in the seroconversion and infection of a significant number of initially uninfected animals, it will serve as a natural infection model to test efficacy of vaccine candidates being developed for elk. This strategy could be thought of as a controlled field trial in confinement. The large cost benefit of using this model would allow greatly accelerated testing of candidate vaccines for brucellosis in elk. Collaborators for this project will include WY Game and Fish and ARS/NADC.

Budget (Travel, Capture, Housing, Feed, 17 months)

Commented [pn6]: Talk to Kreeger

Proposed Long Term:

Elk Genome Project

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Sequencing, assembly, and annotation of the elk genome would greatly support and expand researchers' efforts to explore and understand the immunological responses elicited by this species to *B. abortus* and will aid in development of vaccines against *B. abortus* infection in elk. Additionally, this valuable knowledge will support vaccine and diagnostics development in the context of other elk diseases as well. Collaborators and funders will need to be identified such as ARS/NADC, Texas A and M University, Iowa State University.

Budget (Sequencing Assembly and Annotation)

\$120,000

Will seek collaborators and other support

Brucella vaccine studies in elk and feral swine

Continued exploration of *B. suis* vaccine candidates in feral swine in an experimental infection model using feral swine from a breeding colony established at the APHI/VS animal pens. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Continued exploration of *B. abortus* vaccines in elk. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Budget

To be determined

Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic

Human populations in the Arctic ecosystem are potentially exposed to zoonotic agents through subsistence hunting practices which include raw meat preparation and consumption. Marine mammal *Brucella* spp. and *B. suis* biovar 4 (found in terrestrial animals especially caribou) as well as other agents, such as *Coxiella* spp., exist on the arctic landscape and can cause disease in humans. In the event of a human diagnosed with brucellosis, steps to determine the *Brucella* species involved are generally not taken. Although the epidemiology and ecology of *B. suis* in caribou and reindeer is relatively well understood, we have very limited information on marine mammal *Brucella* species. We intend to submit a grant proposing to investigate the epidemiology of marine mammal *Brucellae* and the role they play in Arctic wildlife health as well as human health. Collaborators: Varied but may include NVSL, CSU, State of AK, USGS, University of AK, Burrow of Barrow, AK, University of Calgary.

Budget

Commented [pn7]: Cost of raising and maintaining pigs

Commented [pn8]: Put in what we ultimately put in the grant?

Ecology of diseases in feral swine in the United States

Feral swine populations throughout the United States are expanding rapidly and certain swine diseases of economic importance are therefore expanding as well within these populations. We propose to work in collaboration with the Wildlife/Livestock Disease Unit of CEAH to investigate ranges and movements of feral swine populations, estimate disease prevalence, evaluate certain disease

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management strategies, such as vaccination, and incorporate acquired information into disease models. Diseases of interest will include those already existing in the feral swine populations (swine brucellosis, tularemia, pseudorabies) as well as FADs (foot and mouth disease, classical swine fever).

FADs

Initiate studies examining the pathogenesis of Rift Valley fever in potentially susceptible North American wildlife such as white-tailed deer, bison, and elk

Budget

To be determined

Evaluate foot and mouth disease vaccines for use in feral swine and white-tailed deer.

Budget

To be determined

International work

Access to *Brucella* and *M. bovis*-infected animals in the United States is limited to experimental infection studies and the occasional outbreak in cattle and captive cervids. Few opportunities arise that allow researchers to collect samples from naturally infected animals in adequate numbers. Collaborations with colleagues in other countries such as Mexico could make relatively large numbers of infected animals accessible for testing breath samples from cattle for *M. bovis* or *B. abortus*-specific VOCs. **Budget**

To be determined

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: WILDIT projects to be forwarded eventually to Beth Lauthner and Larry Granger-please edit as appropriate
Date: Monday, December 10, 2012 11:15:00 AM
Attachments: [WILDIT Current and Proposed Work 12-10-12.docx](#)

Pauline Nol, DVM, MS, PhD
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**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

On going:

- Evaluation of volatile organic compounds and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals **2010-**
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Proposed Short Term

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**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

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Budget:

\$40,000

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Budget (Animal feed and maintenance for 9 months)

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Budget (Travel)

\$1,500

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Working with CSU scientists to identify lipids uniquely produced by *Brucella* species in order to identify *Brucella*-infected animals as well as distinguish the particular species of *Brucella*. This work will lead to the development of ELISA tests to serologically distinguish between *Brucella* spp. and to differentiate them from *Yersinia enterocolitica* 0:9 infection. Collaborators: CSU, USDA/ARS.

Budget

To be determined

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

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Investigate the seroprevalence of *Brucella* spp. antibodies in grizzly bears and polar bears in Alaska and the Greater Yellowstone Area. Data will be obtained from archived samples and samples to be collected during future field capture events. Collaborators: USGS, State of Alaska, NVSL, CEAH, State of MT

NPRB grant being submitted for Alaskan polar bear work

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Budget (Farrowing, maintenance, travel)

\$4,000

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Commented [pn1]: Cost??

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Budget

\$3,500

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Embryo Transfer as disease mitigation strategy in bison

Development of an effective embryo transfer technique in bison in order to produce disease-free offspring derived from bison with various infections such as brucellosis and paratuberculosis. Collaborators: CSU, WCS, NVSL

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Budget (Tissue handling and laboratory)

\$

Commented [pn3]: Cost?

Proposed Short Term

Inactivated *Mycobacterium bovis* vaccine in feral swine

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Budget (Purchase and shipment of feral piglets from Hawaii, Feed and maintenance of animals (non-BSL3), Animal Care, Animal per diem BSL 3, Laboratory/Immunology, Tissue Culture, Histologic Preparation

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Budget (Travel, Capture, Housing, Feed, 17 months)

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Proposed Long Term:

Elk Genome Project

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

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Budget To be determined

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APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013

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FADs

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Budget

To be determined

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Pauline Nol, DVM, MS, PhD
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**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

On going:

- Evaluation of volatile organic compounds and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals **2010-**
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Proposed Short Term

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**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

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Budget (Animal feed and maintenance for 9 months)

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Budget

To be determined

**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic

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Budget (Travel, Feed and Maintenance, Drugs, Darts, Lease)

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\$3,500

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**APHIS/Veterinary Services
Wildlife Livestock Disease Investigations Team
Work Plan FY 2013**

Budget (Tissue handling and laboratory)

\$

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Proposed Short Term

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\$31,150

***Brucella abortus* infection model in elk**

Brucella abortus is extremely expensive and difficult to work with due to its status as a select agent. In addition, experimental infection models in elk have not been very successful in mimicking natural infection events. We propose to develop a natural infection model for *B. abortus* in elk that would allow test animals to be held in outdoor pens, therefore reducing costs as well as stress on the animals that would otherwise be required to be held in BSL-3 facilities. This study would involve capturing 3 year old pregnant *B. abortus*-infected elk from the wild as well as nonpregnant, 2-3 year old uninfected elk. Infected and uninfected elk will be housed in the same double-fenced paddock and infected elk will be allowed to calve and abort in the pen. The uninfected elk will be thus naturally exposed to infected fetuses, placentas, and/or discharges. These exposed elk will be bred and held for another year to monitor whether they seroconvert and abort the following calving season. If this approach results in the seroconversion and infection of a significant number of initially uninfected animals, it will serve as a natural infection model to test efficacy of vaccine candidates being developed for elk. This strategy could be thought of as a controlled field trial in confinement. The large cost benefit of using this model would allow greatly accelerated testing of candidate vaccines for brucellosis in elk. Collaborators for this project will include WY Game and Fish and ARS/NADC.

Budget (Travel, Capture, Housing, Feed, 17 months)

Commented [pn4]: Talk to Kreeger

Proposed Long Term:

Elk Genome Project

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Sequencing, assembly, and annotation of the elk genome would greatly support and expand researchers' efforts to explore and understand the immunological responses elicited by this species to *B. abortus* and will aid in development of vaccines against *B. abortus* infection in elk. Additionally, this valuable knowledge will support vaccine and diagnostics development in the context of other elk diseases as well. Collaborators and funders will need to be identified such as ARS/NADC, Texas A and M University, Iowa State University.

Budget (Sequencing Assembly and Annotation) **\$120,000**

Will seek collaborators and other support

Brucella vaccine studies in elk and feral swine

Continued exploration of *B. suis* vaccine candidates in feral swine in an experimental infection model using feral swine from a breeding colony established at the APHI/VS animal pens. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Continued exploration of *B. abortus* vaccines in elk. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Budget To be determined

Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic

Human populations in the Arctic ecosystem are potentially exposed to zoonotic agents through subsistence hunting practices which include raw meat preparation and consumption. Marine mammal *Brucella* spp. and *B. suis* biovar 4 (found in terrestrial animals especially caribou) as well as other agents, such as *Coxiella* spp., exist on the arctic landscape and can cause disease in humans. In the event of a human diagnosed with brucellosis, steps to determine the *Brucella* species involved are generally not taken. Although the epidemiology and ecology of *B. suis* in caribou and reindeer is relatively well understood, we have very limited information on marine mammal *Brucella* species. We intend to submit a grant proposing to investigate the epidemiology of marine mammal *Brucellae* and the role they play in Arctic wildlife health as well as human health. Collaborators: Varied but may include NVSL, CSU, State of AK, USGS, University of AK, Burrow of Barrow, AK, University of Calgary.

Budget To be determined

Ecology of diseases in feral swine in the United States

Feral swine populations throughout the United States are expanding rapidly and certain swine diseases of economic importance are therefore expanding as well within these populations. We propose to work in collaboration with the Wildlife/Livestock Disease Unit of CEAH to investigate ranges and movements of feral swine populations, estimate disease prevalence, evaluate certain disease

Commented [pn5]: Cost of raising and maintaining pigs

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management strategies, such as vaccination, and incorporate acquired information into disease models. Diseases of interest will include those already existing in the feral swine populations (swine brucellosis, tularemia, pseudorabies) as well as FADs (foot and mouth disease, classical swine fever).

FADs

Initiate studies examining the pathogenesis of Rift Valley fever in potentially susceptible North American wildlife such as white-tailed deer, bison, and elk

Budget

To be determined

Evaluate foot and mouth disease vaccines for use in feral swine and white-tailed deer.

Budget

To be determined

International work

Access to *Brucella* and *M. bovis*-infected animals in the United States is limited to experimental infection studies and the occasional outbreak in cattle and captive cervids. Few opportunities arise that allow researchers to collect samples from naturally infected animals in adequate numbers. Collaborations with colleagues in other countries such as Mexico could make relatively large numbers of infected animals accessible for testing breath samples from cattle for *M. bovis* or *B. abortus*-specific VOCs. **Budget**

To be determined