

From: [Nol, Pauline - APHIS](#)
To: keith.roehr@state.co.us
Cc: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: 1-27 and CVI for bison from MT to CO
Date: Thursday, January 15, 2015 9:55:45 AM

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

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: 1-27
Date: Tuesday, June 30, 2015 7:59:16 AM
Attachments: [ATT00001.txt](#)

ATT00001.txt

Sent from my iPhone

From: [Stephens, Stephanie H - APHIS](#)
To: [Willard, Tracy A - APHIS](#); [Clark, Terry W - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Cc: [Washington, Phillip E - APHIS](#)
Subject: 10/5/2011 Conference Call Notes: GonaCon Bison Tribal Issues
Date: Thursday, October 06, 2011 2:36:04 PM

Hi Terry, Jack, Tracy and Pauline-

Thank you again for participating in the conference call yesterday on tribal consultation issues for the GonaCon bison experiment, I think it was really useful and productive!

This is a quick summary of what we talked about, the actions we agreed to, our respective responsibilities and some proposed deadlines based on the notes I took. Please review and let me know if anything looks incorrect or if you think the proposed deadlines are not possible.

Purpose of Call: To initiate discussions on tribal consultation issues associated with GonaCon bison experiment and associated NEPA document being written by ERAS

Participants: Jack Rhyan, VS Project Principle Investigator
Pauline Nol, VS Project Investigator
Terry Clark, VS Tribal Liaison
Tracy Willard, ERAS NEPA Project Staff
Stephanie Stephens, ERAS NEPA Project Lead

Summary: Terry provided background information to the call participants on the increasing importance of tribal consultation issues in APHIS. The group discussed the design and timing of the GonaCon study. The group also discuss the NEPA and pesticide (FIFRA) aspects and requirements of the project as they related to tribal issues.

Terry raised several issues that he initially thought tribes might be concerned with, including whether the bison from the study would be rereleased into Yellowstone National Park, whether study animals would be available for hunting and eventual human consumption, how tribes might perceive that the proposed study could limit their access to bison, and how tribes' spiritual feelings about bison might be raised in the context of the study. Terry said that he felt some of the potential issues could be discussed and maybe resolved by providing information on the study and the regulatory limitations in place.

Terry recommended that letters with a general summary of the project be written and sent to identified tribes as a first step in communicating on tribal issues. Depending on the responses to the initial letters, a decision can be made on whether a more formal consultation process needs to take place. The group agreed with Terry's recommended approach to send out initial project information to tribes. Terry also proposed that the procedures outlined in the draft Tribal Consultation Directive prepared by the APHIS BPI Tribal Consultation Team (which will be finalized soon) be followed for the GonaCon bison project. The group also agreed with this proposal.

Actions/

Responsibilities: Identify tribes to contact—Terry Clark, by 10/21/11
Write draft letter to tribes summarizing GonaCon bison project—Terry Clark, by 10/21/11
Review and comment on draft tribes letter—Jack Rhyan, Pauline Nol, Stephanie Stephens and Tracy Willard, by 10/26/11
Schedule follow up group conference call to discuss tribal consultation issues status—Stephanie Stephens, week of 10/24/11
Determine who in VS will sign letter—Terry Clark and Jack Rhyan, by 10/26/11
Send letters to tribes—Terry Clark, with assistance as necessary, by 11/4/11
Next steps tbd

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: [Jack C Rhyan](#)
To: [Brian J McCluskey](#)
Cc: [Matt McCollum](#); [Pauline Nol](#)
Subject: A quick idea to push "decreasing prevalence"
Date: Friday, February 05, 2010 3:21:00 PM

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: Jack C Rhyan <jack.c.rhyan@aphis.usda.gov>
Sent: Friday, February 05, 2010 3:21 PM
To: Brian J McCluskey
Cc: Matt McCollum; Pauline Nol
Subject: A quick idea to push "decreasing prevalence"

Brian,

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What are your thoughts?

Jack

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#)
Subject: Abortion!!
Date: Tuesday, January 27, 2015 3:00:15 PM

Already had an abortion. Placenta definitely looks infected. Red 08 at SlipnSlide, had a live birth in 2013, abortion last year.
WOW!

From: [Rhyon, Jack C - APHIS](#)
To: [Herriott, Donald E - APHIS](#)
Cc: [Robbe Austerman, Suelee - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Activities update
Date: Friday, August 23, 2013 4:48:23 PM
Attachments: [WILDITActivitiesUpdateAug2013.docx](#)

Don,

Attached is the activities update for WiLDIT you requested.

Jack

Wildlife/Livestock Disease Investigations Team (WILDIT)

USDA/APHIS/VS, Western Region

Current Projects and Activities Update, August 2013

Projects – Ongoing and planned field/animal work

1. *Brucella abortus* infection/transmission model in elk. Project to start in **winter/early spring 2014**. 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. Collaborators: ARS, State of WY, CSU
2. Boosted vaccination with RB51 in free-ranging bison in Yellowstone National Park; a field trial to determine effectiveness of vaccine at decreasing abortions and shedding. We are proposing this study to determine if the boosted vaccine, previously shown to perform well in containment, is effective in a field setting. **Spring 2014**. Collaborators: ARS, YNP (possibly).
3. Molokai feral swine colony. Establish breeding colony of feral swine originating from Molokai-**winter/early spring 2014**. These swine will be used for the purposes of tuberculosis vaccine studies.
4. Oral feral swine vaccine studies using killed or rough *Brucella suis* vaccines-**Fall 2014**. Collaborators: ARS, CSU, Virginia Tech.
5. Oral feral swine vaccine studies using killed *Mycobacterium bovis* vaccines. **Fall 2014**. Collaborators: ARS, CSU, IREC (Spain).
6. Develop methods for detection of diseases in feral swine using volatile organic compounds in breath and feces. **FY 14 and after**. Collaborators: USDA-WS, CSU, Technion-Israel, ARS
7. Develop methods for detection of diseases in ruminants using volatile organic compounds in breath and feces. **FY 14 and after**. Collaborators: USDA-WS, CSU, Technion-Israel, ARS
8. Development of breath collection device for feral swine in order to remotely detect diseases in swine using volatile organic compounds. **Developmental work ongoing**
9. Oral Fluid sampling of feral swine (*Sus scrofa*): A tool for disease surveillance and management. Investigate passive rope collection device for swine oral fluids. Explore attractants for collection device. **Ongoing**. Collaborators: APHIS-WS, CSU
10. Efficacy of aerosolized killed *Brucella abortus* in mice. Study to determine protection of *B. abortus* killed by various methods when nebulized multiple times to laboratory mice. This study is to collect preliminary data on killing methods of *B. abortus* as a vaccine. Subsequent studies will involve killed *B. abortus* vaccines that are in spray-dried form. **Animal work ongoing**. Collaborators: APHIS-WS, ARS, CSU
11. Develop cost benefit analysis of using the above stated bioeconomic decision model for managing brucellosis in wildlife in the Greater Yellowstone Area. **Discussion/data collection stage**. APHIS-WS; APHIS-VS-CEAH
12. 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. **Animal work ongoing**. Collaborators: APHIS-WS, USGS- BRD, TNC.
13. Develop embryo transfer technology for disease mitigation in bison. **Animal work ongoing (year 3)** Collaborators: CSU, Wildlife Conservation Society
14. 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. **Received North Pacific Research Board grant in 2013 for retrospective and prospective work on *Brucella* spp. in polar bears. Performing Delphi survey on defining polar bear health and research priorities. Performing formal literature review on polar bear health.** Collaborators: USGS-BRD, NVSL, CSU

15. Lipidomics as an antibody-based diagnostic method for brucellosis and bovine tuberculosis. **Applying for grant money for further research based on promising proof of concept data.** Collaborators: CSU, NVSL
16. 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.

Projects – Field/animal work completed, laboratory work, data analysis, and/or manuscript preparations ongoing

1. Develop bioeconomic decision model for disease mitigation at the wildlife livestock interface. A model was collaboratively developed by a team comprised of staff from CEAH, a wildlife disease economist from Wildlife Services, and WiLDIT. **Manuscript in preparation.** Collaborators: APHIS-WS; APHIS-VS-CEAH
2. Oral feral swine vaccine studies using 2 candidate rough *Brucella suis* vaccines. Studies to determine protection of 2 candidate vaccines in feral swine. **Animal portion concluded. Manuscript in preparation.** Collaborators: ARS, CSU, Virginia Tech.
3. 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. **Manuscripts in preparation.** Collaborators: APHIS-WS, ARS, CSU, Israel Institute of Technology.
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. **Manuscript in preparation.** Collaborators: ARS, NVSL, CSU.
5. 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. **Manuscript in preparation.** Collaborators: ARS, CSU, NVSL, Virginia Tech.
6. 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. **Published.** Collaborators: ARS, NVSL, CSU.
7. 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

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.

Hosted eleven student interns/externs and visiting scientists between January 2013 and August 2013.

Publications 2012-2013:

Rhyan, J. C., H. Van Campen, M. McCollum, P. Nol, R. Davis, J. P. Barfield, and M. Salman. 2013. Rabies in two bison from Colorado. *Case Reports in Veterinary Medicine*. 10.1155/2013/90672. .

Commented [pn1]: Might be missing a few?

- Drolet, B. S., L. M. Reister, J. O. Mecham, W. C. Wilson, P. Nol, K. C. VerCauteren, P. A. van Rijn, T. Rigg, and R. A. Bowen. Experimental infection of white-tailed deer with bluetongue virus serotype 8. *Veterinary Microbiology*. *In press*.
- Rhyan, J. C., P. Nol, C. Quance, A. Gertonson, J. Belfrage, L. Harris, K. Straka, and S. Robbe-Austerman. 2013. Transmission of brucellosis from free-ranging elk to ranched cattle and bison herds in the Greater Yellowstone Area, 2002-2012. *Emerging Infectious Diseases*. *In press*
- Rhyan, J.C., L. Miller, and K. Fagerstone. The use of contraception as a disease management tool in wildlife. *Journal of Zoo and Wildlife Management*. *In press*.
- McCollum, M., J. Rhyan, S. Coburn, D. Ewalt, C. Lahr, P. Nol, T. Keefe, C. Kimberling, and M. Salman. Clinical, culture, serology, and histopathology outcomes of bighorn sheep experimentally infected with *Brucella ovis*. *Journal of Wildlife Diseases*. *In press*.
- Pilon, J. L., J. C. Rhyan, L. L. Wolfe, T. R. Davis, M. P. McCollum, K. I. O'Rourke, T. R. Spraker, K. C. VerCauteren, M. W. Miller, T. Gidlewski, T. A. Nichols, L. A. Miller, and P. Nol. 2013 Immunization with a synthetic peptide vaccine fails to protect mule deer (*Odocoileus hemionus*) from chronic wasting disease. *Journal of Wildlife Diseases*. 49: 694-698.
- Uhrig, S., P. Nol, M. McCollum, and M. Salman and J. Rhyan. 2013 Evaluation of transmission of *Brucella abortus* strain 19 in bison by intravaginal, intrauterine, and intraconjunctival inoculation. *Journal of Wildlife Diseases*. 49: 522-526.
- Rhyan, J. 2013. Pathogenesis and pathobiology of brucellosis in wildlife. *Rev. sci. tech. Off. Int. Epiz* 32:127-132.
- Nol, P., Rhyan, J. C., S. Robbe-Austerman, M. P. McCollum, T. D. Rigg, N. T. Saklou, and M. D. Salman. 2013. The potential for transmission of BCG from orally vaccinated white-tailed deer (*Odocoileus virginianus*) to cattle (*Bos taurus*) through a contaminated environment: Experimental findings. *PLOS ONE* 8(4): e60257. doi:10.1371/journal.pone.0060257.
- Peled, N., R. Ionescu, P. Nol, O. Barash, M. McCollum, K. VerCauteren, M. Koslow, R. Stahl, J. Rhyan, and H. Haick. 2012. Detection of volatile organic compounds in cattle naturally infected with *Mycobacterium bovis*. *Sensors and Actuators B: Chemical*. 171-172: 588- 594
- R. K. Frey, P. R. Clarke, J. Rhyan, M. McCollum, P. Nol, K. Aune. Feasibility of Quarantine Procedures of bison (*Bison bison*) calves from Yellowstone National Park for conservation of brucellosis free bison. *JAVMA* in press.
- C. K. Ellis, R. S. Stahl, Pauline Nol, W. R. Waters, M. V. Palmer, J. C. Rhyan, K. C. VerCauteren, M. McCollum, M. D. Salman. Use of Volatile Organic Compound-based Breath Analysis to Differentiate Healthy Cattle from Cattle Experimentally Infected with *Mycobacterium bovis*. Submitted.
- A. Bayn, P. Nol, U. Tisch, J. Rhyan, C. K. Ellis and H. Haick. Detection of volatile organic compounds in bison seropositive for or infected with *Brucella abortus*. In prep.

Census of Animals in Fort Collins Facility

Bison: 29 (Yellowstone genetics); 31 owned & maintained by CSU for embryo transfer project.

Feral swine: 13 (foundation herd for producing pigs for studies)

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#); [Greiner, Steven J - APHIS](#)
Subject: ACUC pertaining to QA 1858
Date: Wednesday, January 04, 2012 2:03:00 PM
Attachments: [ACUCBisonGonaConStudyfinal \(2\).pdf](#)



I will get you the fully signed version as soon as possible

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

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. Pyper

Date

5/16/2011

Concur

IACUC Chair

Date

From: [Jack C Rhyan](#)
To: [Patrick R Clarke](#)
Cc: [Pauline Nol](#); [Rebecca K Frey](#); [Matt McCollum](#)
Subject: ACUC Proposal - signed
Date: Monday, May 16, 2011 12:08:00 PM
Attachments: [ACUCGonaConBisonStudy.pdf](#)

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

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-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black;">Permit(s) description</td> <td style="width: 25%; border-bottom: 1px solid black;">Number</td> <td style="width: 25%; border-bottom: 1px solid black;">Date</td> </tr> </table>	Permit(s) description	Number	Date
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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 GonaConTM 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: 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.

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Xylazine- 0.07 mg/kg, IM dart

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Tolazoline-300 mg as needed IM

Reversal for BAM:

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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.

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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

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PART ONE: SIGNATURE PAGE

Study Director:



Date:

5/16/11

Concur:

IACUC Chair

Date

From: [Rhyan, Jack C. \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#)
Subject: ACUC
Date: Monday, May 23, 2011 4:08:13 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011final.docx](#)

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>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*
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- ☒ *Other: _____ Live animal work _____*

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	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		
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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
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Name	Address	Role in Study
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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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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~~^{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 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 ~~one injection of~~ GonaCon™ vaccine (containing 3000µg in ~~32~~ ml adjuvant) ~~delivered~~ intramuscularly ~~1 ½ mls on either in the right~~ 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), and in summer following calving.~~^{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 ~~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.~~

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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: [Patrick R Clarke](#)
To: [Jack C Rhyan](#); [Pauline Nol](#); [Matt McCollum](#); [Rebecca K Frey](#)
Subject: After the GonaCon call tomorrow.
Date: Monday, April 25, 2011 12:16:00 PM

All,
Could everyone could stay "on" the GonaCon call tomorrow..... (after YNP has disconnected)
....so that we can discuss some Bison Quarantine/MTFWP issues?

Thanks,
Ryan

P. Ryan Clarke, D.V.M.
USDA/APHIS/VS
Regional Epidemiologist- GYA
Belgrade, MT.
(406) 388-5162
(b) (6) -cell

From: [Nol, Pauline \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#)
Subject: amendment document for the IACUC
Date: Friday, July 01, 2011 2:46:00 PM
Attachments: [ACUC Proposal GonaConBisonStudy2011amendmentform7.1.11.docx](#)



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)

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 anestrus 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 anestrus 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 GonaConTM 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: [Nol, Pauline \(APHIS\)](#)
To: [Rhyan, Jack C \(APHIS\)](#)
Subject: amendments to GonaCon protocol
Date: Friday, July 01, 2011 10:48:00 AM
Attachments: [ACUC Proposal GonaConBisonStudy2011amendment7.1.11.docx](#)



Hey Jack,

I've attached the ACUC protocol with suggested amendments based on our conversations with the ES folks and Cat Bens.

Let me know what you think.

We will have to amend this through the IACUC. And once that's done, I will attach both the original and the amendments to the NWRC protocol and submit that.

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)

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>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, 2019

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

Commented [pn1]: According to Cat Bens, we need to establish an MTA with MVDL if we want to send them stuff

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 efficacy of GonaCon™ as an immunocontraceptive in female *Brucella abortus*-infected bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objective:

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 *Brucella abortus*-seropositive female bison will not reduce shedding of *B. abortus* among penmates.
2. Vaccination with GonaCon™ will not reduce pregnancy rates in female *Brucella abortus*-infected bison
3. Immunocontraceptive vaccine-induced prolonged anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

Commented [pn2]: This is what I changed the objectives and hypotheses to

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 (Rhyen 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.

Serologic testing for evidence of *B. abortus* infection in cows, bulls, and calves will be conducted 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 for *Brucella* 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 [pn3]: Again, need MTA to work with MVDL. For NVSL, we will only need a chain of custody form

Serology evaluating antibody production against GnRH will be conducted at the NWRC. Serology will be conducted prior to vaccination.

Commented [pn4]: How often will we collect blood for this?

At the end of the study, all animals seropositive for *B. abortus* 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.

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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.

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.

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.

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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, [Brucella](#)-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 _____ Date_____

Concur

IACUC Chair _____ Date_____

From: [Frey, Rebecca K - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Another long term study question.....
Date: Friday, February 06, 2015 12:05:19 PM

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: another search
Date: Wednesday, March 02, 2011 9:19:00 AM
Attachments: [test substance = GonaCon.rtf](#)

Hi Pauline,

Sorry, I should have also included a search on GonaCon (attached).

Laura

(See attached file: test substance = GonaCon.rtf)

NWRC STUDY RECORDS
(Test Substance = 'GonaCon')
(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
Carlson	1763	Inoculation of European starlings (<i>Sturnus vulgaris</i>) with killed <i>Mycobacterium avian</i> subspecies paratuberculosis
Eisemann	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
Fry	1585	The efficacy of GonaCon in raccoons
	1656	Using hormone antibody levels to evaluate the effectiveness of Gonacon in raccoon pups
Gionfriddo	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
	1633	Field efficacy of GonaCon immunocontraceptive vaccine for contraception of fox squirrels (<i>Sciurus niger</i>) in California
	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
Kemp	1601	Efficacy testing of new GnRH peptide lots, adjuvant formulation changes for GonaCon production, and a novel French Immunocontraceptive protein
Nichols	1791	The effect of the immunocontraceptive GonaCon on chronic wasting disease propagation
O'Hare	1421	Product chemistry: color, physical state, odor, pH, and viscosity - USDA APHIS GonaCon immunocontraceptive vaccine (EPA reg. no. 56228-xx)
Yoder	1382	Effect of GnRH vaccine on black-tailed prairie dogs
	1383	Field efficacy of GonaCon for black-tailed prairie dogs
	1534	Field efficacy of GonaCon for reducing Eastern Grey Squirrel populations
	1563	Transdermal application of a recombinant GnRH vaccine

From: [Keirn, Gail M - APHIS](#)
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
Date: Friday, April 19, 2013 2:32:06 PM
Attachments: [image007.png](#)

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>



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, 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 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; and
- Recovery from the contraceptive treatment and the brucellosis infection can be completed without any further shedding of the bacteria during subsequent pregnancies.

B. Any bulls that seroconvert to positive may, with notification of the National Park Service Key Official, be transferred to an Animal and Plant Health Inspection Service quarantine facility in Fort Collins, Colorado, for a venereal transmission study.

C. 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.

D. 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.

E. 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 19, 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 as described in QA 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. All carcasses, with the exception of those 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 based on serology and culture (blood, milk, swabs) and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.”

Bison that test negative for brucellosis exposure will be:

- Consigned to a quarantine location for further diagnostics;
- Consigned to a managed for public trust conservation program to supplement population genetic diversity;
- 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 any private party that requests transfer of ownership. The Animal and Plant Health Inspection Service will be responsible for organizing the final disposition of the GonaCon™ research animals whether for conservation or transfer to other research.

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. The Animal and Plant Health Inspection Service will notify parties receiving bison of this regulation. Once the bison have left the research facilities, however, the Animal and Plant Health Inspection Service does not have the ability to enforce 36 CFR 10.

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, Montana or Fort Collins, Colorado, 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 research studies at the above specified locations.

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: _____

Daniel N. Wenk
Superintendent, Yellowstone NP
February _____, 2013

Signature: _____

Mark Davidson
Director, Western Region, USDA, APHIS, VS
February _____, 2013

Signature: _____

Tina Holland
Contracting Officer
February _____, 2013

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Bison available for you or slaughter
Date: Thursday, June 04, 2015 9:16:39 AM

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: [Jack C Rhyan](#)
To: [Kathleen A Fagerstone](#); [John D Eisemann/CO/APHIS/USDA](#); [Pauline Nol](#); [Matt McCollum](#); [Lowell A Miller/CO/APHIS/USDA](#)
Subject: bison contraception project
Date: Thursday, December 02, 2010 10:46:00 AM

Kathy et al,
We should meet soon to strategize on the bison project. I'm around mostly til Christmas.
Jack

From: [Jack C. Rhyan](#)
To: [Rebecca K. Frey](#); [Pauline Nol](#); [Matt McCollum](#)
Subject: bison contraception protocol revision
Date: Wednesday, December 01, 2010 5:37:00 PM
Attachments: [ImmunocontBisonProject_12-1.doc](#)

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

(See attached file: ImmunocontBisonProject_12-1.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 (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 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 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, 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. Offspring from seropositive cows will be euthanized and specimens collected for culture when calves are between 8 and 12 months of age. Carcasses will be donated to the Montana Food Bank. Offspring from seronegative cows will be ovariectomized or neutered and the animals provided to Tribes or donated to the Montana Food Bank when calves are between 8 and 12 months of age. 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. When calves are 8 to 12 months of age, donate to MT Food Bank or neuter and donate to Tribes.

Summer 2015 – Euthanize, necropsy and culture study animals, collect ova and semen 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: [Pauline Nol](#)
To: [John D Eisemann/CO/APHIS/USDA](#)
Cc: [Jack C Rhyan](#)
Subject: bison contraception protocol
Date: Tuesday, March 08, 2011 8:31:00 AM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx](#)

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
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pauline.nol@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 <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. <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 .
<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 checked="" type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input checked="" 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 .

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: May 1, 2011

End Date: October 1, 2015

Archive Date: October 1, 2016

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 attached 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: May 1, 2011
Proposed Experimental Termination Date: October 1, 2015
Proposed Study Completion/Archive Date: October 1, 2016

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 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.

Hypotheses:

1. Immunocontraception of *B. abortus*-seropositive female bison will not reduce transmission of *B. abortus* among penmates.
2. immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

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.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Once blocked by serologic status, animals will be randomly selected to go into one of the two pastures (test groups). Seropositive bison in one pasture will receive an injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated. After one year, the vaccinated animals will receive a booster vaccination of 3000µg in order to guarantee maintenance of sterility.

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.

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. 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. 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.

10. Experimental Design and Statistical Analyses

Twenty animals will be assigned to each of two groups. Each group will have at least 10 seropositive cows and 10 seronegative cows. In the treatment group, the ten seropositive cows will be vaccinated with GonaCon (3000µg) to induce sterility, and 10 seronegative cows will share the pasture and be in direct contact with the seropositive cows. In the nontreatment

group, 10 seropositive cows will be vaccinated with adjuvant alone and will share a pasture with 10 seronegative cows. Cows will be exposed to bulls every breeding season and the study will continue through three breeding seasons.

The number of animals to be assigned to the seronegative groups was determined using a power calculation in SAS (power for comparing 2 independent proportions). We consider power to be acceptable at a level of approximately 80%. We will be using a one-sided test and an alpha level of 0.05. The treatment will be deemed successful if the number of seroconversions in the seronegative group exposed to untreated seropositive animals exceeds that of seronegatives exposed to treated seropositives by 50% or more. A sample size of 10 per group was calculated to be sufficient in order to determine differences between treated and untreated groups under the above stated power and alpha constraints. Fisher's Exact Tests will be performed to compare numbers of seroconverted animals in both groups.

10 animals will be assigned to each seropositive group based on previous experience regarding chances that at least one animal (10%) in each group will have the potential to shed *Brucella abortus* via parturition-associated tissues and fluids. 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 transmission 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.

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	

14. Human Health and Safety

HS004-00	Personal protective equipment
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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.

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 et al., 1950;
Rankin, 1965),
Robison et al., 1998
Miller LA, Rhyhan JC, and Drew, M, 2004

Commented [pn1]: Still need to write these out

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): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 45 females, 6 males

Body weight range: 400-1000 kg

Age: 2 year to adult

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: Bison are the target species

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate): The target number of animals in each group is 20, consisting of 10 seropositive animals and 10 seronegative animals. 5 extra seronegative animals will be collected as it is expected that a small percentage of seronegative animals captured will seroconvert during the first year before vaccination.

The study will determine whether there is a difference in the number of seroconversions in naïve animals exposed to *Brucella abortus*-infected animals who are allowed to breed naturally and those who are immunocontracepted with GonaCon. The number of animals to be assigned to the seronegative groups was determined using a power calculation in SAS (power for comparing 2 independent proportions). We consider power to be acceptable at a level of approximately 80%. We will be using a one-sided test and an alpha level of 0.05. The treatment will be deemed successful if the number of seroconversions in the seronegative group exposed to untreated seropositive animals exceeds that of seronegatives exposed to treated seropositives by 50% or more. A sample size of 10 per group was calculated to be sufficient in order to determine differences between treated and untreated groups under the above stated power and alpha constraints.

10 animals will be assigned to each seropositive group based on previous experience regarding chances that at least one animal (10%) in each group will have the potential to shed *Brucella abortus* via parturition-associated tissues and fluids. 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 transmission 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.

C. Source

Animals will be trapped 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 trapped 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

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

Reversal: Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

H. Quarantine

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.

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. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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 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.

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:

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

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).

Commented [pn2]:

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:

Commented [pn3]:

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:

Commented [pn4]:

☐ 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 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 [pn5]: ??

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 [pn6]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Rhyon, Jack C - APHIS](#)
Subject: bison data
Date: Tuesday, May 15, 2012 8:27:39 PM
Attachments: [GonaCon_Animals.pdf](#)



HI,

Here is the data in a pdf. All of the GonaCon vaccinates are at Frank's place. All of the extra negatives are in the far N side of SNS.

The 4 animals left at Brogan's are: Red 10; Red 12; Green 05; Green 18

As for the 4 tomorrow, my 2 cents are of course to put anything negative into the N pasture at SNS. On anything positive, if it is 1: vaccinate and off to Frank's, 2: split between both pens, 3: vaccinate 2 for Frank's, 1 to SNS. You are on your own for suspects!

If any of the green tags are now positives, please change to red eartags that were in the back of Ryan's truck. At least they were before he left.... ☺ If the red tags are negative, we will leave their eartags for now.

Just let me know where everybody went, and have fun....bring water. I will have my cell if you need to call.

Rebecca Frey
Wildlife Biologist/Disease Specialist
Greater Yellowstone Area
USDA APHIS VS
406-333-4425 phone/fax

BANGLE TAG	EARTAG	BACKT	DATE Rec'vd	Sero-stat	Age/DOB	SEX	Gonacon	BLED
Green 01	YNP930740	81VJ65	4/5/2011	NEG	2, 2010	F	N	N
Green 02	YNP930702	81VJ64	3/10/2011	NEG	2, 2010	F	N	Y
Green 03	YNP930731	81VJ65	4/5/2011	NEG	2, 2010	F	N	Y
Green 04	YNP930625	81VJ64	3/8/2011	NEG	3, 2009	F	N	Y
Green 05	YNP930696	81VJ64	3/10/2011	?	2, 2010	F		Y
Green 06	YNP930754	81VJ65	4/5/2011	NEG	2, 2010	F	N	Y
Green 07	YNP930638	81HL60	3/8/2011	NEG	2, 2010	F	N	N
Green 08	YNP930648	81HL60	3/8/2011	NEG	3, 2009	F	N	Y
Green 09	YNP930755	81VJ65	4/5/2011	NEG	2, 2010	F	N	Y
Green 10	YNP930626	81VJ64	3/8/2011	NEG	3, 2009	F	N	Y
Green 11	YNP930675	81VJ64	3/10/2011	NEG	3, 2009	F	N	N
Green 12	YNP930670	81VJ64	3/10/2011	NEG	2, 2010	F	N	N
Green 13	81AJW3732		4/26/2011	NEG	1, 2011	F	N	N
Green 14	YNP930725	81VJ65	3/10/2011	NEG	3, 2009	F	N	Y
Green 15	YNP930634	81HL60	3/8/2011	NEG	2, 2010	F	N	Y
Green 16	81AJW3751		4/26/2011	NEG	1, 2010	F	N	N
Green 17	YNP930627	81VJ64	3/8/2011	NEG	3, 2009	F	N	Y
Green 18	YNP930631	81VJ64	3/8/2011	?	2, 2010	F		Y
Red 01	YNP930472		4/26/2011	POS	2, 2010	F	Y	Y
Red 02	YNP930705	81VJ64	3/10/2011	SUS	2, 2010	F	Y	Y
Red 03	YNP930689	81VJ64	3/10/2011	POS	3, 2009	F	N	Y
Red 04	YNP930759	6048	5/23/2011	POS	3, 2009	F	Y	Y
Red 05	YNP930697	81VJ64	3/10/2011	POS	2, 2010	F	Y	Y
Red 06	YNP930287		4/26/2011	POS	2, 2010	F	N	Y
Red 07	YNP930773	8536	5/23/2011	POS	3, 2009	F	N	Y
Red 08	YNP930761	6050	5/23/2011	POS	3, 2009	F	N	Y
Red 09	YNP930760	6049	5/23/2011	POS	1, 2011	F	N	Y
Red 10	YNP930502		4/26/2011	?	2, 2010	F		Y
Red 11	YNP930777	8541	5/23/2011	POS	2, 2010	F	Y	Y
Red 12	YNP930765	8528	5/23/2011	?	2, 2010	F		Y
Red 13	YNP930737	81VJ65	4/26/2011	POS	2, 2010	F	N	Y
Red 14	YNP930150		4/26/2011	POS	2, 2010	F	Y	Y
Red 15	YNP930706	81VJ64	3/10/2011	POS	2, 2010	F	N	Y
Red 16	YNP930684	81VJ64	3/10/2011	POS	2, 2010	F	N	Y
Red 17	YNP930588		4/26/2011	POS	2, 2010	F	N	Y
Red 18	YNP930776	8540	5/23/2011	POS	3, 2009	F	N	Y
Red 19	YNP930762	8523	5/23/2011	POS	2, 2010	F	Y	Y
Red 20	YNP930678	81VJ64	3/10/2011	POS	3, 2009	F	Y	Y
Red 21	YNP930763	8526	5/23/2011	POS	3, 2009	F	N	Y
Red 22	YNP930673	81VJ64	3/10/2011	POS	3, 2009	F	N	Y
Red 23	YNP930667	81VJ64	3/10/2011	POS	3, 2009	F	Y	Y
Red 24	YNP930636		4/26/2011	POS	3, 2009	F	Y	Y
Red 25	YNP930778	8542	5/23/2011	POS	3, 2009	F	N	Y
Red 26	YNP930202		4/26/2011	POS	3, 2009	F	Y	Y
Red 27	YNP930454		4/26/2011	POS	3, 2009	F	Y	Y
Red 28	YNP930575		4/26/2011	POS	3, 2009	F	Y	Y

Red 29	YNP930406	4/26/2011 POS	3, 2009	F	Y	Y
Red 30	YNP930568	4/26/2011 POS	2, 2010	F	N	Y

OLD EARTAG	Datechngd	Disposition	Deworm
		Xtra	
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		Xtra-calf of Grn 11	
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Frank's
SNS

From: [Pauline Nol](#)
To: [Jeffrey M Kemp/CO/APHIS/USDA](#)
Subject: bison GnRH project
Date: Wednesday, November 03, 2010 12:22:00 PM
Attachments: [ImmunocontBisonProject_10-13.doc](#)

This might have been further modified but you'll get the gist.

(See attached file: ImmunocontBisonProject_10-13.doc)

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
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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 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 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 semi-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 GonaConTM 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). 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. Serology for each of the cows, bulls and calves will be monitored twice a year. 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. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring from seropositive cows will be euthanized and specimens collected for culture when calves are between 8 and 12 months of age. Carcasses will be donated to the Montana Food Bank. Offspring from seronegative cows will be

ovarectomized or neutered and the animals provided to Tribes or donated to the Montana Food Bank when calves are between 8 and 12 months of age.

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 – 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. When calves are 8 to 12 months of age, donate to MT Food Bank or neuter and donate to Tribes.

Summer 2015 – Euthanize, necropsy and culture study animals, collect ova and semen 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: [Powers, Jenny](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Cc: margaret_wild@nps.gov; [Rick Wallen](#)
Subject: Bison gonacon shedding study
Date: Monday, January 14, 2013 1:38:49 PM

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
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jenny_powers@nps.gov

From: [Kelly, Kyle J - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Bison IDs for B Abortus samples
Date: Friday, June 14, 2013 12:28:54 PM
Attachments: [Bison for Jack.xlsx](#)

From: Quance, Christine R - APHIS
Sent: Friday, June 14, 2013 11:30 AM
To: Kelly, Kyle J - APHIS
Cc: Rhyan, Jack C - APHIS; Gertonson, Arnold A - APHIS; Robbe Austerman, Suelee - APHIS
Subject: Bison IDs

Chris Quance
Microbiologist/Team Leader
National Veterinary Services Laboratories
Diagnostic Bacteriology Laboratory
Mycobacteria and Brucella Section
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.r.quance@aphis.usda.gov

Accession	Case #	Submitter	S. City	S. State	Owner	O. City	Owner State	Animal Country	Animal State	Species	Sample ID	Animal ID	Ident if cat on	Year Received	ID	State	County Code	Species	Head Year	Head Lett	Animal Lab	Master P. o Date	Reg cat	Public (V/N)	PATRIC	Source - AZ	SNP Validation	Genotyping Code
602350	B09-0553	Claire, D. Ryan	Belg ade	MT	BQFS	Belg ade	MT	Pa k	MT	B son		85-08	B ucella abo tus b ova 1	09	BAL1	MT	067	BI	WL	x	1			N	N	Sent	Y	B09-0553_09BAL1_MIT-067_BI-WLx1
10023707	B10-0973	Claire, P. Ryan-bull bison study	Belg ade	MT	USDA/APHIS/V5	Belg ade	MT	Gallat n	MT	B son		YNP95021	B ucella abo tus b ova 1	10	BAL1	MT	031	BI	WL	x	8	2/26/2013		N	N		m s d f	B10-0973_10BAL1_MIT-031_BI-WLx1
10023707	B10-0975	Claire, P. Ryan-bull bison study	Belg ade	MT	USDA/APHIS/V5	Belg ade	MT	Gallat n	MT	B son		YNP95023	B ucella abo tus b ova 1	10	BAL1	MT	031	BI	WL	x	8	2/26/2013		N	N	Sent	Y	B10-0975_10BAL1_MIT-031_BI-WLx1
11038123	B11-0442	MT Dept of Livestock	Bozeman	MT	YNP	Mammoth	WY	X	MT	B son		8-456 / P11-016	B ucella abo tus b ova 1	11	BAL1	MT	XXX	BI	WL	x		1/10/2013		N	N	Sent	Y	B11-0442_11BAL1_MIT-XXX_BI-WLx
11019422	B11-0469	MT Dept of Livestock	Bozeman	MT	YNP	Mammoth	WY	X	WY	B son		8-458 / P11-017	B ucella abo tus b ova 1	11	BAL1	MT	XXX	BI	WL	x		1/16/2013		N	N	Sent	Y	B11-0469_11BAL1_MIT-XXX_BI-WLx
11020659	B11-0480	Claire, D. Ryan	Belg ade	MT	Yellowstone Nat onal Pa k Wildlife	West Yellowstone	MT	Gallat n	MT	B son		YNP950043	B ucella abo tus b ova 1	11	BAL1	MT	031	BI	WL	x		1/16/2013		N	N	Sent	Y	B11-0480_11BAL1_MIT-031_BI-WLx
11021534	B11-0488	MT Dept. of Livestock	Bozeman	MT	YNP	Mammoth	WY	X	WY	B son		8-470 / P11-024	B ucella abo tus b ova 1	11	BAL1	MT	XXX	BI	WL	x		1/16/2013		N	N	Sent	Y	B11-0488_11BAL1_MIT-XXX_BI-WLx
11040274	B11-0574	Rhyman, D. Jack	Fo i a Coll rs	CO	USDA	Fo i a Coll rs	CO			B son		711	B ucella abo tus b ova 1	11	BAL1		BI	WL	x			1/16/2013		N	N			B11-0574_11BAL1_BI-WLx
12030298	B12-0460	Rhyman, D. Jack	Fo i a Coll rs	CO	USDA/APHIS/V5	Fo i a Coll rs	CO			B son		017	B ucella abo tus b ova 1	12	BAL1		BI	WL	x		1/3/2013		N	N	Sent	Y	B12-0460_12BAL1_BI-WLx	
12034627	B12-0560	Rhyman, D. Jack	Fo i a Coll rs	CO	USDA/APHIS/V5	Fo i a Coll rs	CO			B son		017	B ucella abo tus b ova 1	12	BAL1		BI	WL	x		1/3/2013		N	N	Sent	Y	B12-0560_12BAL1_BI-WLx	
13005136	13-0061	Claire, D. Ryan	Belg ade	MT	BQFS: B son Qua ant ne Feas b l ty Study	Co w n Sp ngs	MT	Pa k	MT	B son		ed 03	B ucella abo tus b ova 1	13	BAL1	MT	67	BI	WL	x		2/26/2013		N	N	Sent	Y	B13-0061_13BAL1_MIT-67_BI-WLx
559510	08-0783	Claire, D. Ryan	Belg ade	MT	USDA/APHIS/V5 / BQFS	Bozeman	MT	Pa k	MT	B son		9-08 / B1ARG3891	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-0783_08BAL1_MIT-067_BI-WLx
559510	08-0795	Claire, D. Ryan	Belg ade	MT	USDA/APHIS/V5 / BQFS	Bozeman	MT	Pa k	MT	B son		1-08 / B1AYE4039	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-0795_08BAL1_MIT-067_BI-WLx
561953	08-0931	Rhyman, D. Jack	Fo i a Coll rs	CO	Rhyman, D. Jack (Nat W lfr Resea ch Ctr)	Fo i a Coll rs	CO	Pa k	MT	B son		9-08	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-0931_08BAL1_MIT-067_BI-WLx
561953	08-0933	Rhyman, D. Jack	Fo i a Coll rs	CO	Rhyman, D. Jack (Nat W lfr Resea ch Ctr)	Fo i a Coll rs	CO	Pa k	MT	B son		1-08	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-0933_08BAL1_MIT-067_BI-WLx
563110	08-1019	Claire, D. Ryan	Belg ade	MT	USDA/APHIS/V5/BQFS	Bozeman	MT	Pa k	MT	B son		0-08 / B1ARG3869	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-1019_08BAL1_MIT-067_BI-WLx
563110	08-1080	Claire, D. Ryan	Belg ade	MT	USDA/APHIS/V5/BQFS	Bozeman	MT	Pa k	MT	B son		1-08 / B1APM1605	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-1080_08BAL1_MIT-067_BI-WLx
563912	08-1122	Claire, D. Ryan	Belg ade	MT	BQFS	Co w n Sp ngs	MT	Pa k	MT	B son		1-08 / B1APM1605	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-1122_08BAL1_MIT-067_BI-WLx
563912	08-1124	Claire, D. Ryan	Belg ade	MT	BQFS	Co w n Sp ngs	MT	Pa k	MT	B son		0-08 / B1ARG3869	B ucella abo tus b ova 1	08	BAL1	MT	067	BI	WL	x				N	N		Y	B08-1124_08BAL1_MIT-067_BI-WLx
13013440	13-0083	Claire, D. Pat ck R.	Belg ade	MT	USDA/APHIS/V5-BQ-Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 16	Red 16	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	4/25/2013	5-1 Plac	N	N		Y	B13-0083_13BAL1_MIT-067_BI-WLx
13013849	13-0086	MT Dept of Livestock	Bozeman	MT	USDA/APHIS/V5-BQ-Gonacoon	Ga llat ne	MT	Pa k	MT	B son	8-435	Red 16 fetus / bison	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	MT	4/25/2013	5-1 Fet Lung	N	N		Y	B13-0086_13BAL1_MIT-067_BI-WLx
13015343	13-0090	Claire, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	5/9/2013	5-1 Vagfx, 5-2 Vagfsw, 5-3 Placsw	N	N		Y	B13-0090_13BAL1_MIT-067_BI-WLx
13015343	13-009052	Claire, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	5/9/2013	5-1 Vagfx, 5-2 Vagfsw, 5-3 Placsw	N	N		Y	B13-009052_13BAL1_MIT-067_BI-WLx
13015343	13-009053	Claire, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	5/9/2013	5-1 Vagfx, 5-2 Vagfsw, 5-3 Placsw	N	N		Y	B13-009053_13BAL1_MIT-067_BI-WLx
13015343	13-0091	Claire, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	G een 09	G een 09	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	5/9/2013	5-1 Plac, 5-2 M ILC	N	N		Y	B13-0091_13BAL1_MIT-067_BI-WLx
13015343	13-009152	Claire, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacoon	Co w n Sp ngs	MT	Pa k	MT	B son	G een 09	G een 09	B ucella abo tus b ova 1	13	BAL1	MT	067	BI	WL	x	8	5/9/2013	5-1 Plac, 5-2 M ILC	N	N		Y	B13-009152_13BAL1_MIT-067_BI-WLx

From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: bison implants
Date: Monday, February 04, 2013 5:45:22 PM

Update on the radio situation.....well, as of yesterday another green tag cow pooped a radio. So, Brent came down, we got the 2 at SlipnSlide in the chute and replaced the radios....they were still pregnant, so all is well. At Riglers, the one cow must be a narco addict, as I stuck 3 darts in her, and she never even broke stride.....so I finally hit her with a double dose.....and that worked! She was also still pregnant, so we just poked it back in there and called it good. I hope that is the last premature delivery.....:-)

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: bison in January
Date: Monday, November 24, 2014 10:23:16 AM

Hello,

I have 3 negative cows, and 1 that was negative, had a couple suspect tests, then went negative again.....that you can have if you want them. I have more than enough sentinel animals for the control group.

I also have 7-9 calves that you can take, 3 males and 4 females for sure, and maybe a couple more females. I need to confirm with Ryan how many he wants to keep.

Then we need to know what if any bulls or cows or anything from Yellowstone you want. They are maybe planning on not testing and just shipping everything to slaughter, so we need to have our ducks in a row on our needs. That includes what needs sniffed too; so we can get them to at least test a few days for us.

Thanks,
Becky

From: [Jack C Rhyan](#)
To: [Pauline Nol](#); [Matt McCollum](#); [Rebecca K Frey](#); [Patrick R Clarke](#); rick_wallen@nps.gov; margaret_wild@nps.gov; Jenny_powers@nps.gov
Subject: bison proj
Date: Wednesday, May 11, 2011 11:52:00 AM

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: (b) (6)
To: [Nol. Pauline - APHIS](#)
Subject: bison sample log spread sheets
Date: Monday, January 14, 2013 3:01:05 PM
Attachments: [Montana Bison GnRH sample log.xlsx](#)
[San Dune Bison GnRH study QA-1923.xlsx](#)

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(b) (6)
DVM Candidate 2015
M.S. Toxicology
B.S. Environmental Health
Colorado State University
(b) (6) rams.colostate.edu

Montana: Gonacon Bison

	Treatment	May-12	1/9/2013
R01	vax		
R03	control		missing
R02	vax		
R04	vax		
R05	vax		
R06	control		
R07	control		
R08	control		
R09			
R10	vax		missing
R11	vax		
R12	control		missing
R13	control		
R14			
R15	control		
R16	control		
R17	control		
R18	control		
R19	vax		
R20	vax		
R21	control		
R22	control		
R23	vax		
R24	vax		
R25	control		
R26	vax		
R27	vax		
R28	vax		
R29	vax		
R30	control		
R31			
G2			
G3			
G4			
G5			
G8			
G9			
G10			
G14			
G15			
G17			

QA-1923 Bison San Dune's

ID	TXT	Nov-11	Nov-12
1	vax	yes	
2	vax	yes	yes
3	vac	yes	yes
4	vax	yes	
5	vax	yes	yes
6	vax	yes	yes
7	vax	yes	yes
8	vax	yes	yes
9	vax	yes	
10	vax	yes	yes
11	control	yes	
12	control	yes	
13	control	yes	
14	control	yes	yes
15	control	yes	
16	control	yes	
17	control	yes	
18	control	yes	yes
19	control	yes	yes
20	control	yes	yes

From: [Rhyon, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: keith.roehr@state.co.us; carl.heckendorf@state.co.us; [Linfield, Thomas F - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: bison ship on 127
Date: Wednesday, June 17, 2015 3:34:59 PM

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: [Jenny Powers@nps.gov](mailto:Jenny_Powers@nps.gov)
To: [Jack Rhyan](#); [pauline nol](#)
Subject: bison study
Date: Tuesday, May 10, 2011 1:11:00 PM

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: [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: Bison to NWRC
Date: Wednesday, August 20, 2014 3:47:39 PM
Attachments: [MT HC 81-454415 Bison to NWRC.pdf](#)

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

Montana Department of Livestock
State Veterinarian
PO Box 202001, Helena, MT 59620-2001

MONTANA
CERTIFICATE OF VETERINARY INSPECTION

81 **454415**
TO ACCOMPANY SHIPMENT

CONSIGNOR NAME AND ADDRESS APHIS, VS, Genal Con Conwin Springs, MT		CONSIGNEE NAME AND ADDRESS APHIS, VS, NWRC 4101 LaPorte Ave Ft Collins, Co. 80521		PERMIT NO. 20KX08-01	DATE ISSUED 20 Aug 14
ORIGIN ADDRESS (IF DIFFERENT THAN ABOVE)		DESTINATION ADDRESS (IF DIFFERENT THAN ABOVE)		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 FREE <input type="checkbox"/> TB MODIFIED ACCREDIT <input type="checkbox"/> BRUCELLOSIS FREE <input type="checkbox"/> PRV STAGE V <input checked="" type="checkbox"/> OTHER: DSA		CARRIER: <input checked="" type="checkbox"/> TRUCK <input type="checkbox"/> OTHER: NAME & ADDRESS: APHIS VS 4101 LaPorte Ave Ft. Collins, CO 80521	
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		ORIGIN OF SHIPMENT: A) County: Park B) Market:		VACCINATION OR TREATMENT FOR (EXCEPT BRUCELLOSIS) PRODUCT: _____ DATE: _____ RECORD NEGATIVE TEST RESULTS LAB: _____	
EAR TAG NO. TATTOO OR OTHER PERMANENT IDENTIFICATION		REGISTRATION NAME AND NUMBER OR DESCRIPTION		VACCINATION TATTOO SYMBOL OR DATE	AGE
1 81 ASW 3760		1 Red 65		N/A	14
2 81 ASW 3757		2 Red 69		14	M
3 81 ASW 3774		3 Red 61		14	Bison
4 YNP 930781		4 Red 63		14	
5 YNP 930786		5 Red 66		24	
6 YNP 930797		6 Red 59		24	
7 YNP 930798		7 Red 62		24	
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: **[Signature]**
Printed Last Name: **CLARKE** License # **1081**
Address: **[Redacted]** **(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: **[Signature]**
Address: **[Redacted]** **(b) (6)**
Date: **20 Aug 14**

From: [Nol, Pauline - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Held, Karl E - APHIS](#); [Bartlett, Justin H - APHIS](#)
Subject: bison tomorrow (Tuesday) morning 8am
Date: Monday, January 26, 2015 10:39:00 AM

Hey there,

I can't remember if I sent out an email last week, but the plan is to work the west pen bison (the 10? youngsters brought from Montana last year) to collect blood for brucella titers/culture. These data will be useful for the Gonacon study and will also serve as baseline for the RB51 dart study.

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: [Rhyon, Jack C - APHIS](#)
To: [Davidson, Mark L - APHIS](#); [Herriott, Donald E - APHIS](#)
Cc: [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#); rick_wallen@nps.gov; [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: bison transfer agreement
Date: Tuesday, February 19, 2013 4:05:44 PM
Attachments: [APHIS_BisonTransferAgreement_19Feb2013.docx](#)

Mark and Don,

We have worked through this document among ourselves and with our Yellowstone colleagues. This is a draft we have agreed on. It is an agreement to allow us to receive bison from the trap this spring for the second cohort of the contraception study. Please review it. We're hoping to get signatures on it this week as the bison will likely be in the trap shortly.

Thanks much,

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, 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 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; and
- Recovery from the contraceptive treatment and the brucellosis infection can be completed without any further shedding of the bacteria during subsequent pregnancies.

B. Any bulls that seroconvert to positive may, with notification of the National Park Service Key Official, be transferred to an Animal and Plant Health Inspection Service quarantine facility in Fort Collins, Colorado, for a venereal transmission study.

C. 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.

D. 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.

E. 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 19, 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 as described in QA 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. All carcasses, with the exception of those 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 based on serology and culture (blood, milk, swabs) and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.”

Bison that test negative for brucellosis exposure will be:

- Consigned to a quarantine location for further diagnostics;
- Consigned to a managed for public trust conservation program to supplement population genetic diversity;
- 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 any private party that requests transfer of ownership. The Animal and Plant Health Inspection Service will be responsible for organizing the final disposition of the GonaCon™ research animals whether for conservation or transfer to other research.

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. The Animal and Plant Health Inspection Service will notify parties receiving bison of this regulation. Once the bison have left the research facilities, however, the Animal and Plant Health Inspection Service does not have the ability to enforce 36 CFR 10.

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, Montana or Fort Collins, Colorado, 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 research studies at the above specified locations.

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: _____
Daniel N. Wenk
Superintendent, Yellowstone NP
February ____, 2013

Signature: _____
Mark Davidson
Director, Western Region, USDA, APHIS, VS
February ____, 2013

Signature: _____
Tina Holland
Contracting Officer
February ____, 2013

From: [McCollum, Matthew P - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#); [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: Bison working plan
Date: Monday, May 07, 2012 2:32:36 PM

Hi all,

So the plan is to work the GnRH bison next week. We (Jack, Pauline?, and I) will drive up on Monday, we'll bleed, swab, and sniff them and hopefully be done in a day, but it might take Tuesday and Wednesday. So we'll come down Wednesday or Thursday.

Matt

From: [Jack C Rhyan](#)
To: [Brian J McCluskey](#); [Patrick R Clarke](#); [Rebecca K Frey](#); [Lowell A Miller/CO/APHIS/USDA](#); [jeff kemp](#)
Cc: [Pauline Nol](#); [Matt McCollum](#)
Subject: brief protocol for bison immunocontraceptive project
Date: Wednesday, June 16, 2010 12:36:00 PM
Attachments: [ImmunocontBisonProject.doc](#)

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

(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:

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?

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: [Patrick R. Clarke](#); [Rebecca K. Frey](#); [Pauline Nol](#); [Matt McCollum](#)
Subject: brief protocol of immunocontraceptive study
Date: Wednesday, October 13, 2010 3:23:00 PM
Attachments: [ImmunocontBisonProject_10-13.doc](#)

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(*See attached file: ImmunocontBisonProject_10-13.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 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 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 semi-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). 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. Serology for each of the cows, bulls and calves will be monitored twice a year. 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. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring from seropositive cows will be euthanized and specimens collected for culture when calves are between 8 and 12 months of age. Caracsses will be donated to the Montana Food Bank. Offspring from seronegative cows will be

ovarectomized or neutered and the animals provided to Tribes or donated to the Montana Food Bank when calves are between 8 and 12 months of age.

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 – 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. When calves are 8 to 12 months of age, donate to MT Food Bank or neuter and donate to Tribes.

Summer 2015 – Euthanize, necropsy and culture study animals, collect ova and semen 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: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: bulls serology from 12/13
Date: Wednesday, January 04, 2012 4:49:01 PM



In case you did not get an email or fax from DOL lab on our GonaCon animals.....I can send it, but thought you should know that the bull was much hotter than last test. 130.5FPA, 3+160CF and+200Rivanol

Rebecca Frey

Wildlife Biologist/Disease Specialist
Greater Yellowstone Area
406-333-4425

From: [Rhyon, Jack C - APHIS](#)
To: kmcdonald@mt.gov
Cc: [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: bulls
Date: Monday, March 26, 2012 3:55:53 PM

Ken,

How are things? Better, I hope, after the dust settles.

As you know, we are planning a study to start at the old quarantine feasibility study site this spring. We need 4 seronegative bulls for that study. We had 4 from last year's capture but they all seroconverted after we got them. I spoke with Dave Hunter this morning and he said he'd be glad to send us 4 from the previous quarantine animals that are now at Turner's. He said he'd like to get them out anyway because they have dominated the breeding so far.

We'd like to use them in our study and if they remain seronegative, we will return them to the state. If not, they would need to go to slaughter and we could try to replace them from our study animals that pass quarantine.

We won't need them until June or early July so hopefully the injunction against moving bison will be over. Do you see a problem with this or do you think it will work?

Jack

From: (b) (6) [colostate.edu](mailto:(b)(6)@colostate.edu)
To: [Nol, Pauline - APHIS](#)
Subject: Case Report / Owner: None Provided / Ref: 421 / F1532360*Final 1 / Submitter: Rhyan, Jack
Date: Friday, May 15, 2015 2:22:34 PM
Attachments: [F1532360_Final_1.PDF](#)

Please find attached your reports from the Veterinary Diagnostic Laboratories at Colorado State University.

This email account is not monitored continuously and if you use the reply button it may take a while for us to respond.

If you need immediate assistance please contact the lab at (970) 297-1281

Laboratory Report Final

*This report supersedes all
previous reports for this case*

Case #: F1532360
Referral #: 421
Date Collected:
Date Received: 04/23/2015
Case Coordinator: Dr. Tawfik Aboellail
Owner: None Provided

Email To: pauline.nol@aphis.usda.gov
NWRC/Vet Services
Dr. Pauline Nol
4101 Laporte Ave.
Fort Collins, CO 80521

**Electronically Signed and Authorized
By:**
Dr. Tawfik Aboellail
sent by Cindy Arrieta
on 5/15/2015 2:21:54PM

Case Contacts

Bill To	NWRC/National Wildlife Research Center	970-266-6140	JACK.C.RHYAN@APHIS.USDA.GOV
Report To	Nol, Pauline	970-266-6126	pauline.nol@aphis.usda.gov
Report To	Bahr, Michelle		michelle.1.bahr@APHIS.usda.gov
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

Specimen Details

ID	Taxonomy	Sex	Age
421	American Bison	Female	

Owner: None Provided

Specimens Received: Blood; Body; Brain Tissue; Tissue Pool;

Clinical History

Bison found dead on morning of 4/23/15 – autolyzed, tissues friable, unclotted blood.

Laboratory Findings/Diagnosis

DIAGNOSIS: Multifocal, acute, mild, ulcerative stomatitis with cheek papillary necrosis.

COMMENTS: PCR against bovine herpes virus 2, malignant catarrhal fever is positive. PCR for anthrax was negative on pooled lung tissue and ear notch. Also, rabies FA testing was negative.

GROSS NECROPSY: A head of a young bison was submitted for necropsy. The palatine mucosa was multifocally ulcerated and there was bilateral focal necrosis of cheek papillae.

HISTOPATHOLOGY: Several sections of brain are examined. No significant histologic lesions were present in these sections of brain from cerebellum, brainstem, mid-brain, and cerebrum.

Tawfik A. Aboellail, BVSc, MVSc, PhD, DACVP
Dictated: 4/27/15 TAA
Full report: 5/12/15 TAA Imj

Virology

Rabies FA

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0004	Brain Tissue	24-Apr-2015	Negative

B S L 3

Bacillus anthracis (Anthrax) real-time PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	2	Blood	24-Apr-2015	Negative
421	3	Tissue Pool	24-Apr-2015	Negative Lung and Ear notch pool

M o l e c u l a r D i a g n o s t i c s

Caprine Herpesvirus (CapHV-1) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0005	Tissue Pool	28-Apr-2015	Negative Cheek mucosa, lymph node & lung were pooled for testing.

Ovine Herpesvirus 2 (OHV-2 MCF) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0005	Tissue Pool	27-Apr-2015	Positive Cheek mucosa, lung and lymph node were pooled for testing.

N e c r o p s y

Necropsy Wildlife / Exotics Gross Examination Only

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	1	Body	15-May-2015	Complete

End of Report

From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: CC about excess calves
Date: Monday, November 24, 2014 11:12:57 AM

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: [Eisemann, John D. \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Rhyan, Jack C. \(APHIS\)](#)
Cc: [Stephens, Stephanie H. \(APHIS\)](#); [O'Hare, Jeanette R. \(APHIS\)](#)
Subject: comments on bison protocol
Date: Monday, June 06, 2011 11:03:14 AM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 6 3 11 eisemann comments.docx](#)

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

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 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 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~~2017

Archive Date: October 10, 2019

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~~ This study is not part on 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
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: October 1, 2019

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

[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 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.

I know of two studies where GonaCon was used in Bison. These were straight 'lab' efficacy studies.

- A few years ago Jack and Lowell tested it in a few bison in the VS pens south of NWRC.
- A few years ago Lowell sent TREK zoo GonaCon for use in their collection animals.

Formatted: List Paragraph, Bulleted + Level: 1 + Aligned at: 0.25" + Indent at: 0.5"

Talk to Lowell and Jack about data from these studies. They should be included in the background to show that GonaCon has potential in bison.

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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.

Commented [jde4]: 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)

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.

Commented [jde5]: You mention in the ACP Appendix that animals will be boosted at one year. If this is the plan, you will need to mention it here.

Part of the EUP, will be to say how much test substance will be used in the study and when it will be applied

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 (Rhyen 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.

Commented [jde6]: It would be good to include a detailed timeline for all these activities (June 2012-2017)

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.

Commented [jde7]: NWRC will conduct ELISA tests to determine anti-GnRH titers

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.

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 [jde8]: 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

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

	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 [pn9]:

Commented [jde10]: 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

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

Commented [jde11]: How long with this take? Will care during transport be necessary? What care?

This would be an NWRC IACUC type question!

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.

Commented [jde12]: Again an NWRC IACUC type question

Is there an SOP for this? If not, explain how the animals will be cared for

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.

Commented [jde13]: According to the text in the methods, animal carcasses will also be donated to Native American tribes

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 [pn14]: 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 [pn15]:

Commented [jde16]: 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 [pn17]:

Commented [jde18]: 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:

<u>GnRH/KLH Conjugate (1000 µg)</u>	
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
<u>AduVac™ adjuvant</u>	
<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

Commented [jde19]: Make sure you discuss this will Lowell I would like either Jeanette or me to be in that discussion as well

Any deviation from this formula will have implications on future registration/use of this product

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

Commented [jde20]: This is not stated in the methods section

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 [pn21]: ??

Commented [jde22]: You need to talk to Doreen Griffin or Dave Goldade about this

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 [pn23]: ???

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](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Frey, Rebecca K - APHIS](#)
Subject: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan"s?
Date: Monday, February 11, 2013 12:31:36 PM

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: [Frey, Rebecca K - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: Conference call please?
Date: Friday, October 02, 2015 2:27:25 PM

Hi everybody,

Can we please have a conference call regarding the GonaCon animals, planning, facilities etc....next week?

I can make any day and time work. Please check the 5th-9th for a time to talk for an hour, and let me know what works.

Thanks,

Becky

From: [Frey, Rebecca K - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Rhyon, Jack C - APHIS](#); [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: conference call
Date: Friday, December 05, 2014 9:17:24 AM

Hello,

This is your reminder for our Tuesday Dec. 9 conference call regarding the GonaCon project.....and other serious/top secret business no doubt.

The call in number is (b) (6) passcode is (b) (6)

Yak at ya then!

From: [Frey, Rebecca K - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Cc: [Clarke, Patrick R. - APHIS](#)
Subject: confirmation of animals to CO
Date: Wednesday, December 24, 2014 10:53:52 AM

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: [Rebecca K Frey](#)
To: [Jack C Rhyan](#); [Pauline Nol](#); [Matt McCollum](#); [Patrick R Clarke](#)
Subject: contraception editing
Date: Friday, October 08, 2010 7:49:00 AM
Attachments: [ImmunocontBisonProject_rkf.doc](#)

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

(See attached file: ImmunocontBisonProject_rkf.doc)

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 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:

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?

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: [Rhyon, Jack C - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Coop Agreement draft
Date: Friday, February 15, 2013 10:57:58 AM
Attachments: [APHIS_BisonTransferAgreementJRedits_Feb2013\(1\).docx](#)

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. 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

Animal and Plant Health Inspection 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

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 QA 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 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~~ 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 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~~

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~~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 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: ????????? 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: [Rhyon, Jack C - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Coop Agreement draft
Date: Friday, February 15, 2013 10:57:58 AM
Attachments: [APHIS_BisonTransferAgreementJRedits_Feb2013\(1\).docx](#)

How's this?

Jack

From: [Orahood, Darcy S - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Cc: [Eckery, Douglas C - APHIS](#)
Subject: Custom GonaCon for MT bison
Date: Monday, April 21, 2014 4:03:37 PM

Hi Pauline and Jack,

I will be manufacturing GonaCon this week for the Montana bison study and would like to find out who I will be transferring the emulsion to so that it can be hand-mixed. The emulsion will need to be mixed soon after it is made so that it's easier to work with and to prevent any separation from occurring. Will you please let me know who I need to coordinate with so that I can try to plan my week around the manufacturing and hand-off?

Thank you,

Darcy Orahood

Biological Science Technician

National Wildlife Research Center

4101 LaPorte Avenue

Fort Collins, CO 80521

Phone (970) 266-6061

From: [Pauline Nol](#)
To: [Jack C Rhyan](#); [Matt McCollum](#); [Rebecca K Frey](#); [Patrick R Clarke](#)
Subject: draft ACUC protocol
Date: Thursday, May 12, 2011 11:57:00 AM
Attachments: [ACUC Proposal GonaConBisonStudy2011 draft 5.12.11.docx](#)

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

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="border-bottom: 1px solid black; display: inline-block; width: 150px; margin-right: 10px;"></div> <div style="border-bottom: 1px solid black; display: inline-block; width: 150px; margin-right: 10px;"></div> <div style="border-bottom: 1px solid black; display: inline-block; width: 150px;"></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 .
<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
USDA/APHIS/VS Bison Quarantine Feasibility Study	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility

Location		
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).

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?

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 [pn4]: 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.

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, Rhyan JC, and Drew, M, 2004

Commented [pn5]: 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?

☒ 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:

Commented [pn6]:

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:

Commented [pn7]:

☐ 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 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: _____

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: [McCollum, Matthew P - APHIS](#)
To: [Clarke, Patrick R. - APHIS](#); [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: Draft of bison transfer agreement
Date: Monday, February 24, 2014 3:47:57 PM
Attachments: [APHIS_BisonTransferAgreement_Feb2013MM.docx](#)

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

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.

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
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: [Clarke, Patrick R. - APHIS](#)
To: [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#)
Subject: Draft Version of Bison Quarantine Protocol
Date: Wednesday, January 04, 2012 11:40:47 AM
Attachments: [Final Draft-Quarantine Protocol for Bison DEC 23.docx](#)

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

QUARANTINE PROTOCOL FOR BISON
DRAFT REQUIREMENTS FROM THE BISON QUARANTINE FEASIBILITY STUDY GROUP
DECEMBER 2011

6. Procedures for Handling Infected or Restricted Herds

D. Approved Bison Quarantine Facilities

A group or individual may establish an Approved Bison Quarantine Facility (ABQF) to provide testing for brucellosis-exposed bison from Yellowstone and Grand Teton National Parks in order to qualify the animals as brucellosis-free. These facilities may be located in Yellowstone National Park, Grand Teton National Park, or adjacent to the Parks in the adjoining States of Idaho, Montana, or Wyoming. State and Federal animal health officials must approve each facility. State and Federal animal health officials will monitor and assure protocol requirements and BMPs (best management practices) are met. Facility approval is valid for one year and can be reapproved provided all requirements are met.

The Bison Quarantine Feasibility Study (2005-2011) was initiated with sexually immature bison that were followed through puberty, gestation, and parturition. These guidelines are a product of the work completed with only this class of bison.

State and/or Federal animal health officials will select the serological tests (for antibodies to *B. abortus*) to be conducted, establish procedures to account for all animals entering or leaving the ABQF, and supervise all operations. The minimum recommended battery of serological tests employed during initial monthly screening should be the BAPA (or RAP), the FPA and the CF.

All bison entering an approved bison quarantine facility are considered to be brucellosis-exposed animals and must be permanently identified with official metal eartags and RFID tags and placed under quarantine restrictions. Each bison must have at least two permanent forms of identification prior to entering an Individual Test Group (ITG). Prior to entering the facility, all animals must test negative on official brucellosis serological tests conducted at the National Veterinary Services Laboratories (NVSL) or at an approved Cooperative State-Federal Brucellosis Laboratory (CSFBL). All serological and/or milk tests conducted in the ABQF are considered preliminary and must be confirmed at NVSL or at an approved CSFBL. Specimens or milk samples for bacterial culture must also be cultured at NVSL or at an approved CSFBL.

All test-negative bison captured during a single season entering the ABQF should be placed in an ABQF holding pen until they can be sorted and penned separately into individual test groups (ITGs). The holding pens and ITG pens should be separated by at least two fences that are a minimum of 10 feet apart. Upon entry into the ABQF, it is recommended that serological tests be conducted on every bison every 30-45 days while they are in a holding pen or ITG until each animal classified as a reactor has been removed and the remaining animals test negative. If the testing results in any bison being classified as a reactor, a

subsequent ITG test must be conducted on the remaining animals in the ITG at least 30-45 days later.

Initially, this procedure will more readily identify reactor animals, minimizing the time spent in the ABQF completing the testing requirements to qualify for quarantine release.

All *Brucella* culture-positive animals and/or all animals classified as reactors must be removed from the ABQF within 15 days of being identified. Any bison removed from the ABQF before completing the requirements to qualify for quarantine release must move under permit either to an approved research facility or to an approved slaughter facility for slaughter only. All bison that are classified as reactors because they tested positive to an official serological and/or milk test or are confirmed culture-positive must go to an approved research facility or to an approved slaughter facility for slaughter only.

Each ITG must qualify for quarantine release following the procedures listed below before any individual bison within the ITG may be released from quarantine.

1) Males

- (a) Male bison must pass a minimum of three consecutive negative ITG tests. The interval between tests should be a minimum of 30 days. The first ITG test must be conducted when the ITG starts the quarantine period. A final two negative tests should be conducted after the bull has reached 30 months of age with the final test being conducted after the end of the rutting season (August-October). There must be at least 6 months between the first and last consecutive negative tests.

2) Breeding bulls

- (a) Bulls must be tested negative for brucellosis within 30 days prior to being placed within an ITG for breeding purposes or be from an ITG that has qualified for a quarantine release.

3) Females-Sexually mature (3 years of or older)

- (a) Nonpregnant female bison not born in the facility and continually penned within a test-negative ITG must be bred in the ITG to a test-negative male from a holding pen or ITG, complete a gestation cycle, calve, and pass a minimum of three consecutive negative ITG tests.

The first ITG test must be conducted when the ITG starts the quarantine period before being bred. Testing will continue every 30-45 days until all bison in the ITG that seroconvert are removed. Another test will be conducted 30 days after the last reactor animal was removed. An ITG test must be conducted at least 30 days and not more than 60 days before expected start of calving, and the final ITG test must be conducted six months after the last animal has calved in the ITG. There must be at least 12 months between the first and last consecutive negative ITG tests.

(b) Pregnant female bison must not be accepted for quarantine

4) Females-Sexually immature (under 3 years of age)

(a) Immature female bison under three years of age not born in the facility and continually penned within a test-negative ITG must be bred to a test-negative male from a holding pen or ITG, complete a gestation cycle, calve, and pass a minimum of three consecutive negative ITG tests.

The first ITG test must be conducted when the ITG starts the quarantine period before being bred. Testing will continue every 30-45 days after female bison are at least 18 months of age, until all bison in the ITG that seroconvert are removed. Another ITG test will be 30 days after the last reactor animal was removed. An ITG test must be conducted at least 30 days and not more than 60 days before the expected start of calving, and the final ITG test must be conducted six months after the last animal has calved in the ITG. There must be at least 12 months between the first and last consecutive negative ITG tests.

5) Calves

(a) Calves born in the ABQF from a test- and/or culture-negative ITG of adult pregnant females may be released from quarantine at six months of age or older provided that all of the following conditions are met: 1) there have been no reactor animals in the ITG immediately after their birth or within one month prior to their birth; 2) all calves in the ITG are serologically test-negative; 3) each adult in the ITG is serologically test-negative at 6 months post calving and 4) the adult animals in the ITG have tested negative on three consecutive herd tests over a 12 month period.

6) Test- and/or culture-positive animals or animals that die in quarantine

(a) Any aborted fetus, stillborn animal, or an animal that dies in the ABQF for any reason, will be necropsied, serologically tested, and its tissues and other appropriate specimens cultured for *Brucella*. Tissue collection methods should be based on the sampling protocol outlined by the Greater Yellowstone Interagency Brucellosis Committee (GYIBC). Any culture and/or serologically test-positive animal found in an ITG will cause the ITG to restart the quarantine requirements. Restarting the quarantine requires the ITG to be tested every 30-45 days until all animals classified as reactors have been removed from the ITG and a complete ITG test is negative. Bred female bison in the ITG that have been pregnancy checked and determined not be pregnant must be euthanized, necropsied, and specimens collected and cultured. In addition, a complete epidemiologic assessment will be made of all test or culture-positive cases of brucellosis within the ABQF. All *Brucella* culture-positive animals and/or all animals classified as reactors must be removed from the ABQF within 15 days of being identified. They must be neutered, slaughtered, or moved to an approved research facility.

7) Neutered animals

(a) Neutered bison may be released from quarantine without restrictions.

8) Post-quarantine requirements

(a) The State Animal Health Authorities in the State of destination must authorize movement into their State.

	Minimum tests required to release	Minimum test intervals	Minimum quarantine periods
Males	3	1st: start of quarantine period 2nd: 30-45 days apart Last: after the first rut after reaching 30 months of age.	30 months
Nonpregnant sexually mature females	3	1st: before bred 2nd: 30-45 day intervals 3 rd : between 30 and 60 days before females begin calving Last: 6 months after last animal has calved	1 ½ years
Immature females	3	1st: before bred 2nd: 30-45 days apart, after 18 months of age 3 rd : between 30 and 60 days before calving begins Last: 6 months after last animal has calved	2 ½ years
Calves* (Born at ABQF)	1	One test at 6 months of age	½ year

* Calves born to females that were pregnant upon entry into the ABQF and calves born in an ITG in which reactors have been disclosed shall not be released as calves. They shall be placed in an ITG as an immature female/male.

From: [Nol, Pauline - APHIS](#)
To: [Greiner, Laura B - APHIS](#)
Subject: electronic copy of QA1858
Date: Wednesday, January 04, 2012 12:04:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 12_29_11.docx](#)



Sorry did not send earlier.

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

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 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 (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 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 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 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. 002177

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:

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:

Jack C
Rhyan/CO/APHIS/USDA
02/05/2010 03:23 PM

To Brian J McCluskey/CO/APHIS/USDA
cc Matt McCollum/CO/APHIS/USDA@USDA, Pauline
Nol/CO/APHIS/USDA@USDA
bcc
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 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: [Miller, Lowell A \(APHIS\)](#)
Cc: [Rhyan, Jack C \(APHIS\)](#); [Eisemann, John D \(APHIS\)](#)
Subject: filling in gaps in GonaCon Bison Protocol
Date: Monday, June 06, 2011 3:35:00 PM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 6 3 11 eisemann commentspnrevision 6.6.docx](#)



Lowell,

Could you address the costs for NWRC section as well as information for the Test, Control and Reference Material/Devices Formulation and Use Appendix? I just took it directly from the elk study as a start so it probably is not entirely appropriate for this study.

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)

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 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 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 .

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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~~2017

Archive Date: ~~October 10, 2019~~

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~~ 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
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, 2019⁷
 Proposed Study Completion/Archive Date: October 1, 2019

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

[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 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.

I know of two studies where GonaCon was used in Bison. These were straight 'lab' efficacy studies.

- A few years ago Jack and Lowell tested it in a few bison in the VS pens south of NWRC.
- A few years ago Lowell sent TREK zoo GonaCon for use in their collection animals.

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Talk to Lowell and Jack about data from these studies. They should be included in the background to show that GonaCon has potential in bison.

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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 and 20). 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

Commented [jde4]: 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)

Commented [jde5]: You mention in the ACP Appendix that animals will be boosted at one year. If this is the plan, you will need to mention it here.

Part of the EUP, will be to say how much test substance will be used in the study and when it will be applied.

Commented [pn6]: This has been corrected. No boosting will occur.

Commented [jde7]: It would be good to include a detailed timeline for all these activities (June 2012-2017)

Commented [jde8]: 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 [jde9]: 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.

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 [pn10]:

Commented [jde11]: 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

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. The Corwin Springs facility is within

Commented [jde12]: How long with this take? Will care during transport be necessary? What care?

This would be an NWRC IACUC type question!

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.

Commented [jde13]: Again an NWRC IACUC type question

Is there an SOP for this? If not, explain how the animals will be cared for

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 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 [pn14]: 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 [pn15]:

Commented [jde16]: 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 [pn17]:

Commented [jde18]: 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:

<u>GnRH/KLH Conjugate (1000 µg)</u>	
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
<u>AduVac™ adjuvant</u>	
<i>Mycobacterium avium</i> (Mycopar™ – <i>M. a. paratuberculosis</i>)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

Commented [jde19]: Make sure you discuss this will Lowell I would like either Jeanette or me to be in that discussion as well

Any deviation from this formula will have implications on future registration/use of this product

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.~~

Commented [jde20]: This is not stated in the methods section

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 [pn21]: ??

Commented [jde22]: You need to talk to Doreen Griffin or Dave Goldade about this

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 [pn23]: ???

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](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: Final Draft
Date: Tuesday, January 03, 2012 12:28:39 PM
Attachments: [Final Draft-Quarantine Protocol for Bison DEC 23.docx](#)

Please take a final look at this draft.....if no changes I will pass it onto Drs. McCluskey/Herriott

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

QUARANTINE PROTOCOL FOR BISON
DRAFT REQUIREMENTS FROM THE BISON QUARANTINE FEASIBILITY STUDY GROUP
DECEMBER 2011

6. Procedures for Handling Infected or Restricted Herds

D. Approved Bison Quarantine Facilities

A group or individual may establish an Approved Bison Quarantine Facility (ABQF) to provide testing for brucellosis-exposed bison from Yellowstone and Grand Teton National Parks in order to qualify the animals as brucellosis-free. These facilities may be located in Yellowstone National Park, Grand Teton National Park, or adjacent to the Parks in the adjoining States of Idaho, Montana, or Wyoming. State and Federal animal health officials must approve each facility. State and Federal animal health officials will monitor and assure protocol requirements and BMPs (best management practices) are met. Facility approval is valid for one year and can be reapproved provided all requirements are met.

The Bison Quarantine Feasibility Study (2005-2011) was initiated with sexually immature bison that were followed through puberty, gestation, and parturition. These guidelines are a product of the work completed with only this class of bison.

State and/or Federal animal health officials will select the serological tests (for antibodies to *B. abortus*) to be conducted, establish procedures to account for all animals entering or leaving the ABQF, and supervise all operations. The minimum recommended battery of serological tests employed during initial monthly screening should be the BAPA (or RAP), the FPA and the CF.

All bison entering an approved bison quarantine facility are considered to be brucellosis-exposed animals and must be permanently identified with official metal eartags and RFID tags and placed under quarantine restrictions. Each bison must have at least two permanent forms of identification prior to entering an Individual Test Group (ITG). Prior to entering the facility, all animals must test negative on official brucellosis serological tests conducted at the National Veterinary Services Laboratories (NVSL) or at an approved Cooperative State-Federal Brucellosis Laboratory (CSFBL). All serological and/or milk tests conducted in the ABQF are considered preliminary and must be confirmed at NVSL or at an approved CSFBL. Specimens or milk samples for bacterial culture must also be cultured at NVSL or at an approved CSFBL.

All test-negative bison captured during a single season entering the ABQF should be placed in an ABQF holding pen until they can be sorted and penned separately into individual test groups (ITGs). The holding pens and ITG pens should be separated by at least two fences that are a minimum of 10 feet apart. Upon entry into the ABQF, it is recommended that serological tests be conducted on every bison every 30-45 days while they are in a holding pen or ITG until each animal classified as a reactor has been removed and the remaining animals test negative. If the testing results in any bison being classified as a reactor, a

subsequent ITG test must be conducted on the remaining animals in the ITG at least 30-45 days later.

Initially, this procedure will more readily identify reactor animals, minimizing the time spent in the ABQF completing the testing requirements to qualify for quarantine release.

All *Brucella* culture-positive animals and/or all animals classified as reactors must be removed from the ABQF within 15 days of being identified. Any bison removed from the ABQF before completing the requirements to qualify for quarantine release must move under permit either to an approved research facility or to an approved slaughter facility for slaughter only. All bison that are classified as reactors because they tested positive to an official serological and/or milk test or are confirmed culture-positive must go to an approved research facility or to an approved slaughter facility for slaughter only.

Each ITG must qualify for quarantine release following the procedures listed below before any individual bison within the ITG may be released from quarantine.

1) Males

- (a) Male bison must pass a minimum of three consecutive negative ITG tests. The interval between tests should be a minimum of 30 days. The first ITG test must be conducted when the ITG starts the quarantine period. A final two negative tests should be conducted after the bull has reached 30 months of age with the final test being conducted after the end of the rutting season (August-October). There must be at least 6 months between the first and last consecutive negative tests.

2) Breeding bulls

- (a) Bulls must be tested negative for brucellosis within 30 days prior to being placed within an ITG for breeding purposes or be from an ITG that has qualified for a quarantine release.

3) Females-Sexually mature (3 years of or older)

- (a) Nonpregnant female bison not born in the facility and continually penned within a test-negative ITG must be bred in the ITG to a test-negative male from a holding pen or ITG, complete a gestation cycle, calve, and pass a minimum of three consecutive negative ITG tests.

The first ITG test must be conducted when the ITG starts the quarantine period before being bred. Testing will continue every 30-45 days until all bison in the ITG that seroconvert are removed. Another test will be conducted 30 days after the last reactor animal was removed. An ITG test must be conducted at least 30 days and not more than 60 days before expected start of calving, and the final ITG test must be conducted six months after the last animal has calved in the ITG. There must be at least 12 months between the first and last consecutive negative ITG tests.

(b) Pregnant female bison must not be accepted for quarantine

4) Females-Sexually immature (under 3 years of age)

(a) Immature female bison under three years of age not born in the facility and continually penned within a test-negative ITG must be bred to a test-negative male from a holding pen or ITG, complete a gestation cycle, calve, and pass a minimum of three consecutive negative ITG tests.

The first ITG test must be conducted when the ITG starts the quarantine period before being bred. Testing will continue every 30-45 days after female bison are at least 18 months of age, until all bison in the ITG that seroconvert are removed. Another ITG test will be 30 days after the last reactor animal was removed. An ITG test must be conducted at least 30 days and not more than 60 days before the expected start of calving, and the final ITG test must be conducted six months after the last animal has calved in the ITG. There must be at least 12 months between the first and last consecutive negative ITG tests.

5) Calves

(a) Calves born in the ABQF from a test- and/or culture-negative ITG of adult pregnant females may be released from quarantine at six months of age or older provided that all of the following conditions are met: 1) there have been no reactor animals in the ITG immediately after their birth or within one month prior to their birth; 2) all calves in the ITG are serologically test-negative; 3) each adult in the ITG is serologically test-negative at 6 months post calving and 4) the adult animals in the ITG have tested negative on three consecutive herd tests over a 12 month period.

6) Test- and/or culture-positive animals or animals that die in quarantine

(a) Any aborted fetus, stillborn animal, or an animal that dies in the ABQF for any reason, will be necropsied, serologically tested, and its tissues and other appropriate specimens cultured for *Brucella*. Tissue collection methods should be based on the sampling protocol outlined by the Greater Yellowstone Interagency Brucellosis Committee (GYIBC). Any culture and/or serologically test-positive animal found in an ITG will cause the ITG to restart the quarantine requirements. Restarting the quarantine requires the ITG to be tested every 30-45 days until all animals classified as reactors have been removed from the ITG and a complete ITG test is negative. Bred female bison in the ITG that have been pregnancy checked and determined not be pregnant must be euthanized, necropsied, and specimens collected and cultured. In addition, a complete epidemiologic assessment will be made of all test or culture-positive cases of brucellosis within the ABQF. All *Brucella* culture-positive animals and/or all animals classified as reactors must be removed from the ABQF within 15 days of being identified. They must be neutered, slaughtered, or moved to an approved research facility.

7) Neutered animals

(a) Neutered bison may be released from quarantine without restrictions.

8) Post-quarantine requirements

(a) The State Animal Health Authorities in the State of destination must authorize movement into their State.

	Minimum tests required to release	Minimum test intervals	Minimum quarantine periods
Males	3	1st: start of quarantine period 2nd: 30-45 days apart Last: after the first rut after reaching 30 months of age.	30 months
Nonpregnant sexually mature females	3	1st: before bred 2nd: 30-45 day intervals 3 rd : between 30 and 60 days before females begin calving Last: 6 months after last animal has calved	1 ½ years
Immature females	3	1st: before bred 2nd: 30-45 days apart, after 18 months of age 3 rd : between 30 and 60 days before calving begins Last: 6 months after last animal has calved	2 ½ years
Calves* (Born at ABQF)	1	One test at 6 months of age	½ year

* Calves born to females that were pregnant upon entry into the ABQF and calves born in an ITG in which reactors have been disclosed shall not be released as calves. They shall be placed in an ITG as an immature female/male.

From: [Nelson, Janell - APHIS](#)
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
Date: Friday, February 24, 2012 12:31:35 PM
Attachments: [APHIS FOIAAppeal GonaCon 2-23-2012.pdf](#)
[12-01625 \(Response\) Letter.pdf](#)
[APHIS FOIAreq GonaCon 2-2012.pdf](#)
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



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

(b) (6)

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 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,

(b) (6)

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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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,

(b) (6)

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

002218



UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.
PLANT INDUSTRY REPORT NO. 100

PLANT INDUSTRY REPORT NO. 100
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

PLANT INDUSTRY REPORT NO. 100

PLANT INDUSTRY REPORT NO. 100

The following report was prepared by the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C., for the purpose of providing information regarding the plant industry of the United States.

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PLANT INDUSTRY REPORT NO. 100

The following report was prepared by the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C., for the purpose of providing information regarding the plant industry of the United States.

The following report was prepared by the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C., for the purpose of providing information regarding the plant industry of the United States.

PLANT INDUSTRY REPORT NO. 100

-----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,

(b) (6)

--

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. ***

From: [Nelson, Janell - APHIS](#)
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
Date: Tuesday, January 10, 2012 5:00:00 PM
Attachments: [12-01161.pdf](#)
[image001.png](#)

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:

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: [Nelson, Janell - APHIS](#)
To: [McCluskey, Brian J - APHIS](#); [Herriott, Donald E - APHIS](#); [Clarke, Patrick R. - APHIS](#); [Rhyon, 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
Date: Wednesday, February 08, 2012 9:47:24 AM
Attachments: [12-01470.pdf](#)
[image001.png](#)

Drs. McCluskey, Herriott, Clarke, & Rhyon, 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).

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: 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.

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, Room 4B02.9 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:



Darrell Geist
<z@wildrockies.org>
02/06/2012 06:27 PM

To FOIA Officer/MD/APHIS/USDA
cc <z@wildrockies.org>
bcc
Subject FEBRUARY 6 2012 FREEDOM OF INFORMATION ACT
REQUEST

1 attachment



P1D1278DE 6 1 2 1 2.png

FOIA Request # 12-01470
Date Rec'd 2/6/2012
Date Due 3/6/2012
Assigned to Reggie
Category all other
Search WS



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

February 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. All quarterly, semi-annual and final financial reports for the USDA APHIS Montana Department of Livestock Bison Cooperative Agreement #11-9730-0124-CA.
2. Bison Operation Cooperative Agreement for the time period January 1, 2012 through December 31, 2012.
3. A current agreement or MOU between APHIS and Montana Department of Livestock on the use of APHIS employees or contractors to pilot aircraft in Montana.
4. A current inventory of any APHIS Federally-owned or Federally-leased equipment on loan to the Montana Department of Livestock.
5. Please include all correspondence in the agency's possession on items 1 through 5 above.

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: [Nelson, Janell - APHIS](#)
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
Date: Thursday, March 22, 2012 9:45:47 AM
Attachments: [12-01942.pdf](#)

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 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 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: 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.**



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

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

March 20, 2012

Director Tonya Woods
Freedom of Information & Privacy Act
USDA-Animal & Plant Health Inspection Service
4700 River Road, Unit 50
Riverdale, MD 20737-1232

12-01942
FOIA Request #
Date Rec'd 3/26/2012
Date Due 4/18/2012
Assigned to Melinda
Category All others
Search US/WS

RE: FREEDOM OF INFORMATION ACT REQUEST

Dear Director Woods,

On behalf of Buffalo Field Campaign I am submitting a request pursuant to the Freedom of Information Act, 5 U.S.C. § 552 for an electronic copy of public records on the buffalo removed from Yellowstone National Park by USDA-Animal & Plant Health Inspection Service (USDA-APHIS) for the proposed "Evaluation of GonaCon" study.

To reduce costs and waste of resources, Buffalo Field Campaign asks USDA-APHIS to provide and disclose the following records in electronic form on CD:

1. The specific number of buffalo or bison originally removed from the Yellowstone National Park wild populations and transferred to USDA-APHIS during May 2011 for the proposed "Evaluation of GonaCon" study, or for any other purpose and what, specifically, those purposes are.
2. The number of viable buffalo or bison currently being held by USDA-APHIS for the proposed "Evaluation of GonaCon" study.
3. Records of any buffalo or bison deaths, injuries or other incidents that have incurred, to date, since USDA-APHIS acquired the buffalo or bison for the proposed "Evaluation of GonaCon" study.
4. Specific records of any and all buffalo or bison handling, testing, ear-tagging, collaring, telemetry implants, sorting, vaccinating, medications administered, pregnancies (viable or failed), feeding schedules, quantities and types of feed distributed, water availability for bison held by USDA-APHIS for purpose of the proposed "Evaluation of GonaCon" study.
5. List of the sex and age of all bison acquired by USDA-APHIS for the proposed "Evaluation of GonaCon" study for bison
6. Specific location, sizes, dates used, natural forage availability and water availability of pastures being used by USDA-APHIS for the proposed "Evaluation of GonaCon" study.
7. Specific number, age and sex of buffalo or bison in each pasture being held by USDA-APHIS for the proposed "Evaluation of GonaCon" study.

Records will include any letters, faxes, notes, proposals, reports, plans, permits, video footage, photos, analysis, impact statements, correspondence including emails and attachments, if any. **All correspondence should go through Attorney Daniel C. Snyder, Law Offices of Charles M. Tebbutt, P.C., 451 Blair Blvd., Eugene, Oregon, 97402; (phone) 541-344-3505; (email) dan.tebbuttlaw@gmail.com.**

Buffalo Field Campaign requests a waiver of fees as "disclosure of the information 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 the commercial interest of the requester."

Full disclosure of records will shed light on the operations and activities of USDA-APHIS and its proposed "Evaluation of GonaCon" study.

The records requested will help provide information of how the proposed "Evaluation of GonaCon" study impacts our environment and the public concern/public trust.

Full disclosure fosters Buffalo Field Campaign and our members' ability to engage decision makers, the public, Congress, among others, on a federal decision impacting wild American bison in the ecosystem.

Full disclosure of records requested pursuant to the Freedom of Information Act fosters public access to and knowledge of the proposed "Evaluation of GonaCon" study and such records will inform dialogue amongst the public, decision makers, members of Congress, among others, on a federal decision impacting wild American bison in the ecosystem.

Buffalo Field Campaign is a nonprofit organization incorporated in the state of Montana in 1997 whose projects educate and involve the public in finding solutions to the Yellowstone buffalo slaughter. As part of our mission to involve and educate the American people in important federal decisions impacting the Yellowstone buffalo herd's historic and native range, Buffalo Field Campaign actively develops, promotes and supports programs involving a diverse and broad section of the public at large. These programs include community presence in Gardiner and West Yellowstone, Montana, and outreach through our web site, online news and list serve capabilities that reach tens of thousands of people who, through their involvement, have demonstrated significant concern over the government's actions and activities impacting our country's last wild buffalo herd. Buffalo Field Campaign seeks disclosure of these records for their educational, scientific and informative values.

Buffalo Field Campaign has demonstrated a capability to reach out to and include the public at large and a broad audience of people who have an active interest and concern for American bison and the ecosystems wild populations depend upon for survival. Buffalo Field Campaign programs reach and involve the public in Yellowstone National Park gateway communities, at large in all 50 states, and abroad in various countries, through our newsletter, web site, email list serve, public events outreach and programmatic capabilities.

Fully disclosing these records through a fee waiver to Buffalo Field Campaign will contribute significantly to the public's understanding of the USDA-APHIS proposed "Evaluation of GonaCon" study and its impacts on wild American bison and the ecosystem upon which they depend.

Buffalo Field Campaign has no commercial interest in the records requested, and the waiver of fees sought is consistent with the public interest criteria of the Freedom of Information Act.

To expedite disclosure of records by USDA-APHIS please contact me if I can be of help in any way.

(b) (6)

Buffalo Field Campaign
P.O. Box 957
West Yellowstone, MT 59758
bfc-media@wildrockies.org

cc: Daniel C. Snyder, Attorney at Law, Law Offices of Charles M. Tubbett, P.C.



Buffalo Field Campaign
<BFC-Media@wildrockies.org>

03/20/2012 08:03 PM

To FOIA Officer/MD/APHIS/USDA
cc (b) (6) gmail.com>

bcc

Subject *Freedom of Information Act Request

1 attachment



APHIS FOIA 3-20-12_buffalo info.pdf

Meinda
VS/WS
all other

Greetings Director Woods,

Attached is a Freedom of Information Act Request from Buffalo Field Campaign.

Please be advised that all correspondence in regards to this FOIA request should go through our attorney, Daniel C. Snyder at the following:

Daniel C. Snyder
Law Offices of Charles M. Tebbutt, P.C.
451 Blair Blvd.
Eugene, OR 97402
Ph: 541-344-3505
(b) (6) gmail.com

Thank you for your prompt attention to this request.

Sincerely,

(b) (6)

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. ***

Watch and Share: "Protect the Wild Bison"
<http://www.youtube.com/BFCMEDIA#p/a/f/0/joSrf7Qi7Cw>

Endangered Status of Wild American Buffalo
<http://www.buffalofieldcampaign.org/habitat/bisonconservation.html>

Bison Abuse Merits Harsh Criticism
<http://wolves.wordpress.com/2011/02/17/bison-abuse-merits-harsh-criticism/>

BOYCOTT BEEF! It's what's killing wild buffalo
<http://www.buffalofieldcampaign.org/actnow/boycott.html>

Find BFC on Facebook

<http://www.facebook.com/buffalowild>

From: Nol, Pauline - APHIS
Sent: Tuesday, November 28, 2017 2:59 PM
To: Rhyan, Jack C - APHIS (Jack.C.Rhyan@aphis.usda.gov)
Subject: FW: bison
Attachments: Critical Brucellosis research in MT and CO being.docx; ATT00001.htm

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: Herriott, Donald E - APHIS
 Sent: Friday, February 09, 2018 7:32 AM
 To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
 Subject: FW: Bozeman Chronicle -- seeking comment on FOIA lawsuit

Good morning Jack and Pauline

I chatted with Pauline about this and told her I would send it to you.

Have a great weekend,

Don

From: Hayden, Joelle R - APHIS
 Sent: Thursday, February 8, 2018 11:21 AM
 To: Clarke, Patrick R. - APHIS <Patrick.R.Clarke@aphis.usda.gov>; Herriott, Donald E - APHIS <Don.E.Herriott@aphis.usda.gov>
 Subject: FW: Bozeman Chronicle -- seeking comment on FOIA lawsuit

Hi Ryan and Dr. Herriott,

We were forwarded this media inquiry (that came in by way of our FOIA team). We're running down information - and in all likelihood (b) (5) But we wanted to check to see if you had any information/situational awareness to share.

Thanks,
 Joelle

From: (b) (6) [mailto:(b) (6)@dailychronicle.com]
 Sent: Thursday, February 08, 2018 12:40 PM
 To: Henson, Terry A - APHIS <Terry.A.Henson@aphis.usda.gov>
 Subject: Bozeman Chronicle -- seeking comment on FOIA lawsuit

Hi Terry -- I'm writing today about a lawsuit against APHIS from the Buffalo Field Campaign over delays on a FOIA request they've filed. I'm wondering if APHIS has any comment on the lawsuit or the allegations therein. The complaint can be read at this link: <http://www.buffalofieldcampaign.org/images/news-and-media/press-releases/press-releases-2017-2018/2018-02-08/BFC-vs-USDA-APHIS-Complaint-Filed-02-07-2018.pdf>

My deadline is 5p.m. mountain time. Thanks.

Michael Wright
 Reporter|Bozeman Daily Chronicle
 Office: 406-582-2638 | Cell: (b) (6)

From: Nol, Pauline - APHIS
 Sent: Wednesday, January 24, 2018 3:57 PM
 To: Rhyan, Jack C - APHIS (Jack.C.Rhyan@aphis.usda.gov)
 Subject: FW: Draft Memo
 Attachments: VS Memo_Request to transport bison to research facility_12-6-16_DRAFTmm.doc

This was the letter I was trying to come up with this morning.

From: McCollum, Matthew P - APHIS
 Sent: Tuesday, December 13, 2016 11:29 AM
 To: Linfield, Thomas F - APHIS <Thomas.F.Linfield@aphis.usda.gov>
 Cc: Frey, Rebecca K - APHIS <Rebecca.K.Frey@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
 Subject: RE: Draft Memo

Hey Tom,

First of all, I really appreciate you helping on this. However, in my opinion, (b) (5)

I made a few comments to that effect on the memo.

Can we appeal to Marty along those lines?

Matt

From: Frey, Rebecca K - APHIS
 Sent: Tuesday, December 13, 2016 10:04 AM
 To: McCollum, Matthew P - APHIS <Matt.McCollum@aphis.usda.gov>; Rhyan, Jack C - APHIS <Jack.C.Rhyan@aphis.usda.gov>; Nol, Pauline - APHIS <Pauline.Nol@aphis.usda.gov>
 Subject: Fwd: Draft Memo

Sent from my iPhone

Begin forwarded message:
 From: "Linfield, Thomas F - APHIS" <Thomas.F.Linfield@aphis.usda.gov>
 Date: December 6, 2016 at 6:20:32 PM MST
 To: "Clarke, Patrick R. - APHIS" <Patrick.R.Clarke@aphis.usda.gov>, "Frey, Rebecca K - APHIS" <Rebecca.K.Frey@aphis.usda.gov>, "Hughes, Janet A - APHIS" <Janet.A.Hughes@aphis.usda.gov>
 Subject: Draft Memo
 Ryan, Becky, Janet:

Here is a rough, 1st Draft for a memo/letter we may eventually consider sending to MZ. Not intended for further distribution at this time. For now, I highlighted &/or underlined some of the applicable language in the MCA or ARM sections... Please feel free to provide edits and/or fill in some of the missing info!

Tom

Thomas F.T. Linfield, DVM | Assistant Director | District 5 Field Office for MT | USDA-APHIS-VS
 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: Rhyan, Jack C - APHIS
 Sent: Wednesday, January 24, 2018 12:06 PM
 To: McCluskey, Brian J - APHIS
 Cc: Nol, Pauline - APHIS
 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: 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
 Sent: Monday, November 27, 2017 10:17 AM
 To: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS; Wehtje, Morgan E - APHIS
 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: [Clarke, Patrick R. - APHIS](#)
To: [Frey, Rebecca K. - APHIS](#); [Rhyan, Jack C. - APHIS](#); [Nol, Pauline - APHIS](#); [McCollum, Matthew P. - APHIS](#)
Subject: FW: «ALERT» High Priority NVSL Report - Accession#14-033434,Purpose GEN_DIAG,Exam Req BRUC sent to John.B.Belfrage@aphis.usda.gov,Patrick.R.Clarke@aphis.usda.gov,Debra.A.Donch@aphis.usda.gov,Arnold.A.Gertonson@aphis.usda.gov,Mark.Camacho@aphis.usda.gov
Date: Tuesday, November 04, 2014 11:25:45 AM
Attachments: [14-033434_DBL-BRUC_ALT_10-24-2014-12-24-13-PM.pdf](#)

Interesting to note they cultured B abortus from a hepatic LN on this bison haven't seen that very often

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: Friday, October 24, 2014 11:29 AM
To: Belfrage, John B - APHIS; Clarke, Patrick R - APHIS; Donch, Debra A - APHIS; Gertonson, Arnold A - APHIS; Camacho, Mark S - APHIS; Robbe Austerman, Suelee - APHIS; Quance, Christine R - APHIS
Subject: «ALERT» High Priority NVSL Report - Accession#14-033434,Purpose:GEN_DIAG,Exam Req:BRUC sent to John B Belfrage@aphis.usda.gov,Patrick R Clarke@aphis.usda.gov,Debra A Donch@aphis.usda.gov,Arnold A Gertonson@aphis.usda.gov,Mark Camacho@aphis.usda.gov,

Submitter Name: Amy Boerger-Fields

Submitter Company: University of WY

WY State Veterinary Lab

Referral Number:

FAD Number:

Accession: 14-033434

Date Received: 10/14/2014 09:42:31 AM

Purpose: General Diagnostic

Exam(s) Requested: BRUC

Submitter State: WY

Owner State: WY

Animal State: WY

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

Antler's Ranch

Meeteetse, WY

Animal Location

Park County WY

Submitter - 24991

Amy Boerger-Fields

University of WY

WY State Veterinary Lab

1174 Snowy Range Rd

Laramie, WY 82070

FAX #: 307-721-2051

Phone #: 307-766-9931

Accession Number:

14-033434

Date Collected:

10/06/2014

Date Received:

10/14/2014

Date Completed:

10/24/2014

Collected By:

WY State Vet Lab

Purpose:

General Diagnostic

Referral Number:

This is not a billable case.

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

Sample: N17446 **Animal ID:** 83VAV1801/ Red1 **Brucella Case Number:** B14-0673 **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- Not Identified

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Retropharyngeal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Lymph Node / Lymph Node- Prescapular

Brucella Isolation Result

No Isolation Made

Lymph Node / Lymph Node- Hepatic

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus biovar 1

Tonsil / Tonsil

Brucella Isolation Result

No Isolation Made

Uterus/Vagina / Uterus

Brucella Isolation Result

No Isolation Made

Tissue / Cervix

Brucella Isolation Result

No Isolation Made

Mammary Gland / Mammary Gland

Brucella Isolation Result

No Isolation Made

Mammary Gland / Mammary Gland

Brucella Isolation Result	No Isolation Made
Mammary Gland / Mammary Gland	
Brucella Isolation Result	No Isolation Made
Mammary Gland / Mammary Gland	
Brucella Isolation Result	No Isolation Made
Spleen / Spleen	
Brucella Isolation Result	Suspect Isolated

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.

USDA-APHIS-VS-WR
406-388-5162

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: 4 green 14 abortions results from MDOL
Date: Monday, March 17, 2014 12:29:53 PM
Attachments: [4 Green 14 abortion feb 2014.pdf](#)

From: Clarke, Patrick R. - APHIS
Sent: Monday, March 17, 2014 10:59 AM
To: Frey, Rebecca K - APHIS; Rhyan, Jack C - APHIS
Subject: 4 green 14 abortions results from MDOL

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



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 # 8-324-14
Owner: USDA, APHIS, VS

Species: WILD - BISON
Breed: BISON
Name/No. 4 GREEN 14
Age: FETUS Sex:

Date Sent: 03/10/2014
Date Received: 02/24/2014

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

Final Report

Case Coordinator: AWL

CASE SUMMARY

REASON FOR SUBMISSION: Brucella seropositive cow, abortion

LABORATORY DIAGNOSIS:

Bronchopneumonia: Etiology - Brucella abortus biovar 1

A. W. Layton, DVM, DACVP\cto

Date In 02/27/2014

PATHOLOGY

Date Out: 03/10/2014 Released by: AWL

GROSS: The carcass of a bison fetus that is hairless with approximately 40 cm crown/rump length. The fetus is fair post mortem and nutritional state. The sex was not determined. Lungs are atelectatic. Scant amount of tan fluid occurs in the forestomachs and abomasum. No other significant changes were present.

HISTOPATHOLOGY: Tissue sections of liver, spleen, lung, thymus, abomasum, adrenal gland, lymph node, heart, kidney, skeletal muscle, small intestine and brain are examined. There is a bronchopneumonia, and many large airways contain columnar and squamous epithelial cells. Inflammatory cell component is moderate in number and occurs within alveolar spaces. The infiltrate consists of alveolar macrophages and fewer neutrophils. Fibrin exudation is present in some areas.

MORPHOLOGIC DIAGNOSIS:

Bronchopneumonia, with meconium and squamous inhalation

Date In 02/25/2014

BACTERIOLOGY

Date Out: 03/07/2014 Released by: MH

Isolate sent to NVSL for full identification 2/28/14; results received 3/7/14; identified as Brucella abortus biovar 1.

CULTURES

ID/Site	Specimen	Culture Type	Isolate	Antimicrobial	
				Growth	Profile
	fetal liver	Brucella	Brucella abortus	1+ P	NA
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	Brucella abortus	2+ P	NA
	fetal lung	Brucella	Brucella abortus	2+ P	NA

1+ to 4+ = rare colony to confluent growth

P = pure culture, M = mixed or partially contaminated culture

Date In: 02/24/14

SEROLOGY

Date Out: 03/03/14

Released by: AF

Testname	# of tests	# Negative	Test Summary		# A	C	# Undetermined	# Insufficient	Tech
B. ABORTUS RIVANOL	1	0	# Positive	# Suspect	0		0	0	AF
B. ABORTUS FP	1	0	1	0	0		0	0	AF
B. ABORTUS CF	1	0	1	0	0		0	0	AF
B. ABORTUS CARD	1	0	1	0	0		0	0	AF
B. ABORTUS BAPA	1	0	1	0	0		0	0	AF

List of Significant result

Animal Id	Testname	Result	Titer
4 GREEN 14	B. ABORTUS CARD	POS	
4 GREEN 14	B. ABORTUS CF	POS	4+ 640
4 GREEN 14	B. ABORTUS RIVANOL	POS	+200
4 GREEN 14	B. ABORTUS FP	POS	202.3

Final Classification

Animal Id	Classification	Comment
4 GREEN 14	REACTOR	

Date In: 02/28/2014

REFERRAL/OTHER

Date Out: 03/07/2014

Released by: AVL

Animal ID	Specimen	Test	Result	Rfri Inst.
4 Green 14	Slant Tube	Brucella Culture	Brucella abortus biovar 1	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

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

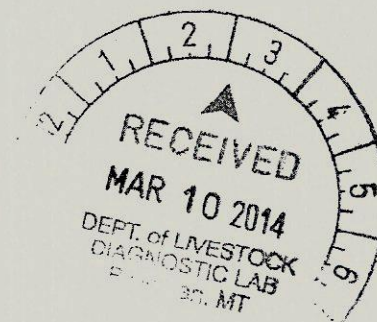
Accession Number: 14-006821
Date Collected: 02/22/2014
Date Received: 03/03/2014
Date Completed: 03/10/2014
Collected By: P. Ryan Clarke
Purpose: General Diagnostic
Referral Number: 8-324-14
This is not a billable case.

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

Sample: 8-324-14 Animal ID: 4 Green 14 / Bison Brucella Case Number: B14-0102 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

Scanned 3/10/14 / JH

Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 70.00
Referral Fee	\$ 18.25
Serology Fee	\$ 9.50
Accession Total Fee	\$ 97.75

(This is not a bill. Do not make payment from this report.)

FEE INCREASE:

Please, note that laboratory fees will increase on October 15, 2013. The Fee Schedule is available on the Laboratory webpage:
<http://www.liv.mt.gov/lab/default.mcp>

If you have any questions, laboratory personnel may be contacted at 406-994-4885 or LIVDiagnosticLab@mt.gov

GonaCon Study Case# 8-324-14

Green#14 Adult Female Bison

This animal is classified a reactor based on positive serological reactions

E. H. Hinkle DVM

Designated Brucellosis Epidemiologist



MT DEPT. OF LIVESTOCK
DIAG. LAB P.O. BOX 997
BOZEMAN, MT 59771-0997
406-994-4885

030514

From: [Rhyan, Jack C \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#)
Subject: FW: amendment document for the IACUC
Date: Monday, July 11, 2011 2:34:02 PM

FYI

From: Stephens, Stephanie H (APHIS)
Sent: Monday, July 11, 2011 1:37 PM
To: Rhyan, Jack C (APHIS)
Subject: RE: amendment document for the IACUC

Good, I'm glad that John was able to talk to you. We had decided last week that it was probably better for John to come find you and speak to you about the whole EUP discussion we had rather than try to put it in an e-mail.

You are correct, the strategy is that we will see if we can get EPA to agree that we don't need an EUP and get that in writing. If that doesn't work, our fallback position will be to do the EUP. We have time to take care of that if needed, so I don't see it as a problem even if EPA says no. Suffice to say we'll make sure you are covered on the FIFRA (EPA) requirements either way.

From: Rhyan, Jack C (APHIS)
Sent: Monday, July 11, 2011 1:31 PM
To: Stephens, Stephanie H (APHIS); Eisemann, John D (APHIS)
Cc: Nol, Pauline (APHIS)
Subject: RE: amendment document for the IACUC

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: [Fagerstone, Kathleen A \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Rhyan, Jack C \(APHIS\)](#)
Subject: FW: BFC press release on Yellowstone bison/contraception
Date: Tuesday, July 05, 2011 8:14:17 AM

I assume you have seen this one.

From: O'Hare, Jeanette R (APHIS)
Sent: Friday, July 01, 2011 10:59 AM
To: Fagerstone, Kathleen A (APHIS); Eisemann, John D (APHIS)
Subject: BFC press release on Yellowstone bison/contraception

FYI – in case you have not seen yet.

<http://www.buffalofieldcampaign.org/media/press1011/pressreleases1011/053111.html>

Jeanette R. O'Hare
Registration Specialist
USDA National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521-2154
970-266-6156 FAX: 970-266-6157

From: [Rhyan, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: BioPRYN Report for Bison & Buffalo
Date: Wednesday, February 05, 2014 12:45:19 PM
Attachments: [LOG 020414001 Rhyan, Dr. Jack - USDA.APHIS.VS 020414001 1391625237774.html](#)

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

Follow us on Facebook and Twitter

BioTracking LLC

1150 Alturas Dr
Suite 105
Moscow, ID 83843
Phone: 208.882.9736
Fax: 208.882.1490
email: biotracking@biotracking.com
web: www.biotracking.com

BioPRYN PSPB Report

Date Received 2/4/2014
Log In # O20414001

Submitted By**USDA/APHIS/VS**

4101 LaPorte Avenue
Fort Collins, CO 80521

Report To

Dr. Jack Rhyan

jack.c.rhyan@aphis.usda.gov

REPORT NOTES:

Mail Report

Tube numbers 47 through 52 also include "Bison" in the tube label.

Report Date

02/05/2014

Assay/Animal

Bison - 52 sample(s)

Number of Samples

52

Open

OD < 0.135

Low Recheck

OD = 0.135 to 0.15

Cutoff

0.15

High Recheck

OD = 0.15 to 0.21

Pregnant

OD > 0.21

Tube Number	Animal ID	Response in Test, OD	PSPB Range	Days Post Breeding
1	G01	0.0696	Open	>40
2	G02	0.9237	Pregnant	>40
3	G03	0.0542	Open	>40
4	G04	0.9237	Pregnant	>40
5	G06	0.9237	Pregnant	>40
6	G07	0.0653	Open	>40
7	G08	0.9237	Pregnant	>40
8	G09	0.0675	Open	>40
9	G10	0.7997	Pregnant	>40
10	G11	0.063	Open	>40
11	G12	0.0632	Open	>40
12	G13	0.0631	Open	>40
13	G14	0.9237	Pregnant	>40
14	G15	0.9237	Pregnant	>40
15	G17	0.9237	Pregnant	>40
16	G18	0.0743	Open	>40
17	R01	0.0707	Open	>40
18	R02	0.9237	Pregnant	>40 (Tube labeled Red 02)
19	R03	0.9237	Pregnant	>40
20	R04	0.0559	Open	>40

002258

21	R05	0.0546	Open	>40
22	R06	0.9237	Pregnant	>40
23	R07	0.9237	Pregnant	>40
24	R08	0.9237	Pregnant	>40
25	R09	0.0719	Open	>40
26	R10	0.0632	Open	>40
27	R11	0.0542	Open	>40 (Tube labeled Red 11)
28	R12	0.0665	Open	>40 (Tube labeled Red 12)
29	R13	0.9237	Pregnant	>40
30	R14	0.0546	Open	>40
31	R15	0.9237	Pregnant	>40
32	R16	0.9237	Pregnant	>40
33	R17	0.0669	Open	>40
34	R18	0.054	Open	>40
35	R19	0.0577	Open	>40
36	R20	0.0683	Open	>40
37	R21	0.9237	Pregnant	>40
38	R22	0.9237	Pregnant	>40
39	R23	0.0751	Open	>40
40	R24	0.9237	Pregnant	>40
41	R25	0.9237	Pregnant	>40
42	R26	0.0587	Open	>40
43	R27	0.0538	Open	>40
44	R28	0.0553	Open	>40
45	R29	0.0586	Open	>40
46	R31	0.0582	Open	>40
47	50	0.0628	Open	>40
48	53	0.0576	Open	>40
49	54	0.0586	Open	>40
50	55	0.0547	Open	>40
51	56	0.0562	Open	>40
52	65-06	0.0557	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 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: [Eisemann, John D. \(APHIS\)](#)
To: [Nol, Pauline \(APHIS\)](#); [Stephens, Stephanie H \(APHIS\)](#)
Subject: FW: bison contraception protocol
Date: Thursday, September 08, 2011 10:47:16 AM
Attachments: [AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx](#)

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
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USDA-APHIS-VS-Western Region
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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:	
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 <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. <div style="border-top: 1px solid black; margin-top: 5px;"> Permit(s) description Number Date </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 .
<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 checked="" type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input checked="" 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 .

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: May 1, 2011

End Date: October 1, 2015

Archive Date: October 1, 2016

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 attached 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: May 1, 2011
Proposed Experimental Termination Date: October 1, 2015
Proposed Study Completion/Archive Date: October 1, 2016

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 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.

Hypotheses:

1. Immunocontraception of *B. abortus*-seropositive female bison will not reduce transmission of *B. abortus* among penmates.
2. immunocontraceptive vaccine-induced prolonged anestrus will have no effect on *B. abortus* colonization in naturally-infected female bison.

9. Methods/Procedures

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.

Bison will be identified with uniquely numbered ear tags and microchip identification.

In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Once blocked by serologic status, animals will be randomly selected to go into one of the two pastures (test groups). Seropositive bison in one pasture will receive an injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated. After one year, the vaccinated animals will receive a booster vaccination of 3000µg in order to guarantee maintenance of sterility.

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.

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. 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. 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.

10. Experimental Design and Statistical Analyses

Twenty animals will be assigned to each of two groups. Each group will have at least 10 seropositive cows and 10 seronegative cows. In the treatment group, the ten seropositive cows will be vaccinated with GonaCon (3000µg) to induce sterility, and 10 seronegative cows will share the pasture and be in direct contact with the seropositive cows. In the nontreatment

group, 10 seropositive cows will be vaccinated with adjuvant alone and will share a pasture with 10 seronegative cows. Cows will be exposed to bulls every breeding season and the study will continue through three breeding seasons.

The number of animals to be assigned to the seronegative groups was determined using a power calculation in SAS (power for comparing 2 independent proportions). We consider power to be acceptable at a level of approximately 80%. We will be using a one-sided test and an alpha level of 0.05. The treatment will be deemed successful if the number of seroconversions in the seronegative group exposed to untreated seropositive animals exceeds that of seronegatives exposed to treated seropositives by 50% or more. A sample size of 10 per group was calculated to be sufficient in order to determine differences between treated and untreated groups under the above stated power and alpha constraints. Fisher's Exact Tests will be performed to compare numbers of seroconverted animals in both groups.

10 animals will be assigned to each seropositive group based on previous experience regarding chances that at least one animal (10%) in each group will have the potential to shed *Brucella abortus* via parturition-associated tissues and fluids. 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 transmission 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.

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	

14. Human Health and Safety

HS004-00	Personal protective equipment
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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.

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 et al., 1950;
Rankin, 1965),
Robison et al., 1998
Miller LA, Rhyhan JC, and Drew, M, 2004

Commented [pn1]: Still need to write these out

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): Bison (Bison bison)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 45 females, 6 males

Body weight range: 400-1000 kg

Age: 2 year to adult

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: Bison are the target species

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate): The target number of animals in each group is 20, consisting of 10 seropositive animals and 10 seronegative animals. 5 extra seronegative animals will be collected as it is expected that a small percentage of seronegative animals captured will seroconvert during the first year before vaccination.

The study will determine whether there is a difference in the number of seroconversions in naïve animals exposed to *Brucella abortus*-infected animals who are allowed to breed naturally and those who are immunocontracepted with GonaCon. The number of animals to be assigned to the seronegative groups was determined using a power calculation in SAS (power for comparing 2 independent proportions). We consider power to be acceptable at a level of approximately 80%. We will be using a one-sided test and an alpha level of 0.05. The treatment will be deemed successful if the number of seroconversions in the seronegative group exposed to untreated seropositive animals exceeds that of seronegatives exposed to treated seropositives by 50% or more. A sample size of 10 per group was calculated to be sufficient in order to determine differences between treated and untreated groups under the above stated power and alpha constraints.

10 animals will be assigned to each seropositive group based on previous experience regarding chances that at least one animal (10%) in each group will have the potential to shed *Brucella abortus* via parturition-associated tissues and fluids. 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 transmission 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.

C. Source

Animals will be trapped 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 trapped 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

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

Reversal: Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given
Tolazoline-300 mg as needed IM

H. Quarantine

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.

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. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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 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.

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:

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

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).

Commented [pn2]:

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:

Commented [pn3]:

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:

Commented [pn4]:

☐ 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 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 [pn5]: ??

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 [pn6]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:

ACP Consultation: _____ Date: _____

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: FW: Bison GonaCon EA - Draft Final Document
Date: Thursday, January 19, 2012 10:00:46 AM
Attachments: [1 18 12 GnRH EA FINAL.docx](#)
[Bison GonaCon LEGAL NOTICE 1 19 12.docx](#)

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

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.

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

⁵Surgical removal of the ovaries from female bison.

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

1. Impact of Proposed Action on Animals

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 as the GnRH antibody titer drops below a protective level.” If the effect of this immunocontraceptive vaccine successfully places the vaccinated

⁶ Surgical removal of the testes of male bison.

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 Hidinger, 1999), may influence their impacts on soil and vegetation.

Bison have a stronger preference for perennial grasses than cattle. Cattle consume a higher percentage of forbs⁸ in their diet than bison, and they

⁷ Steps or shelves in the mountainside that are the remains of former riverbanks or lakeshores.

⁸ Herbaceous flowering plants other than grass.

use wooded areas and riparian zones more intensively than bison (Steuter and Hidinger, 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 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

⁹ When bison roll in shallow depressions in the soil, covering themselves with dirt or mud.

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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LEGAL NOTICE

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is making available to the public an environmental assessment for 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 Yellowstone National Park bison population. This proposed action is planned for locations on private ranch land near Gardiner, Montana. 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," is available online at http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/ and <http://www.ibmp.info>. Paper copies may be obtained by contacting USDA APHIS, Veterinary Services Area Office, 208 North Montana Avenue, Suite 101, Helena, MT 59601 or (406) 449-2220.

Comments may be submitted via email to EAComments2012@aphis.usda.gov or by mail to the VS Area Office listed above. Comments must be received by ~~XX~~, 2012. For more information about the study, please contact the VS Area Office at (406) 449-2220.

Commented [shs1]: date should be 30 days from publication of notice in newspaper

LEGAL NOTICE

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Bison gonacon shedding study
Date: Monday, January 14, 2013 1:39:00 PM

Catchem!

From: Powers, Jenny [mailto:jenny_powers@nps.gov]
Sent: Monday, January 14, 2013 1:39 PM
To: Nol, Pauline - APHIS; Rhyon, 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: [Rhyan, Jack C - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Cc: [Nol, Pauline - APHIS](#)
Subject: FW: Bison gonacon shedding study
Date: Monday, January 14, 2013 1:42:53 PM

Becky,
Park Service agrees.
Thanks.
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

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Jenny Powers, DVM, PhD
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Fort Collins, CO 80525
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(970) 225-3585 (fax)
jenny_powers@nps.gov

From: [Rhyen, Jack C - APHIS](#)
To: [McCollum, Matthew P - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: bison lease APHIS 5/16/12
Date: Friday, May 18, 2012 4:34:23 PM

FY

From: Herriott, Donald E - APHIS
Sent: Friday, May 18, 2012 4:30 PM
To: Santelman, Brenda K - APHIS; Healey, Burke L - APHIS
Cc: Davidson, Mark L - APHIS; Rhyen, Jack C - APHIS; Clarke, Patrick R. - APHIS; Frey, Rebecca K - APHIS; Snyder, Richard W - APHIS; Bartling, David L - APHIS
Subject: RE: bison lease APHIS 5/16/12

Hi Brenda,

After much discussion, we are willing for you to move forward the same rate as the last lease, which I believe was \$50,000 for the year. I know Karin freely worked with others cc'd here to get the most knowledgeable input in the lease negotiations. Please feel free to do likewise.

Thx for all your help,

Don Herriott
Associate Regional Director
970-494-7400

From: Santelman, Brenda K - APHIS
Sent: Wednesday, May 16, 2012 2:04 PM
To: Healey, Burke L - APHIS
Cc: Davidson, Mark L - APHIS; Herriott, Donald E - APHIS
Subject: RE: bison lease APHIS 5/16/12

If you find that a decision won't be made by Friday, just let me know and I'll let (b) (6) know. Thanks!

Brenda Santelman
Realty Specialist/Contracting Officer
USDA, APHIS, MRPBS, Realty Services
100 North Sixth Street
Minneapolis, MN 55403-1588
Ph: 612-336-3231
Fx: 612-336-3553
Brenda.k.santelman@aphis.usda.gov

From: Healey, Burke L - APHIS
Sent: Wednesday, May 16, 2012 3:00 PM
To: Santelman, Brenda K - APHIS
Cc: Davidson, Mark L - APHIS; Herriott, Donald E - APHIS
Subject: RE: bison lease APHIS 5/16/12

Brenda,

I've followed up on this today but will need to get back to Don and Mark. It maybe Friday before they are back and able to discuss options. The 2 options I laid out are still the only two that apply. We , WR VS, need to make a decision on our long range plans for this project. Your reply below is very professional and appropriate.

Thanks,

From: Santelman, Brenda K - APHIS
Sent: Wednesday, May 16, 2012 1:56 PM
To: (b) (6)
Subject: RE: bison lease APHIS 5/16/12

(b) (6),

In my review of the lease file, it appears that the majority of our communications have been via e-mail. If at any time you are needing to call us to request an update, or have questions or concerns, please don't hesitate to do so as our contact information should be on almost every e-mail.

As in any holdover situation, we will continue to pay rent for as long as we are utilizing the property. I have verified with our financial department that you received your March payment in full. Your April payment should be received in your account within a day or two. If we continue to be in holdover status come May, that payment will be issued to you as well.

As we indicated in prior e-mails to you, your current rental proposal is an almost 30 percent increase. This makes your rate to be \$1,181.81/acre, which is not a fair and reasonable rate. During this time of major budget constraints with the Federal budget, we are unable to justify such a significant cost increase. You have since asked for us to provide you a counter offer. As a result, we are diligently reviewing all our options, weighing in the current and upcoming budget cuts we have been asked to make, along with considering the future activities and studies that we will be a part of, in order to provide you a response to our expiring lease.

I should have a response to you by the end of this week. If you have any questions or concerns, please don't hesitate to contact me. My co-worker Karin Finke is out indefinitely, so I will be handling this lease at this time.

Thank you,

*Brenda Santelman
Realty Specialist/Contracting Officer
USDA, APHIS, MRPBS, Realty Services
100 North Sixth Street
Minneapolis, MN 55403-1588
Ph: 612-336-3231
Fx: 612-336-3553*

Brenda.k.santelman@aphis.usda.gov

From: (b) (6) [mailto:(b) (6)@gmail.com]
Sent: Wednesday, May 16, 2012 10:45 AM
To: Santelman, Brenda K - APHIS
Subject: bison lease APHIS 5/16/12

10.204.41.2 with HTTP; Wed, 16 May 2012 08:42:38 -0700 (PDT)
Brenda:

Thank you for the phone call this morning. You are the first live person I have spoken to in many months regarding our leases with APHIS. Just to reiterate we have not been paid since our contract expired 3/16/12 and the APHIS bison have remained on our property since that time. I have in good faith been trying to negotiate a new lease with APHIS well prior to the expiration of our lease.

Please send APHIS's current "good faith" proposal for leasing my property for the stated 6 year contract period to this email.

If APHIS's is no longer going to negotiate with me regarding this contract consider this letter a formal protest.

I look forward to receiving APHIS's response.

Thank you for your attention to this matter.

(b) (6)

From: [McCollum, Matthew P - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Nol, Pauline - APHIS](#)
Subject: FW: Bison preg testing
Date: Tuesday, November 19, 2013 2:57:04 PM

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: [Matt McCollum](#)
To: [Jack C Rhyan](#); Pauline.Nol@aphis.usda.gov
Subject: Fw: bison project
Date: Tuesday, August 10, 2010 10:39:00 AM
Attachments: [In Vitro Fertilization trial with reproductive material from Yellowstone Bison.doc](#)

My quick once-over yielded no issues. You have any heartburn?

M

----- Forwarded by Matt McCollum/CO/APHIS/USDA on 08/10/2010 10:38 AM -----

(b) (6) @gmail.com>

To: Matt.McCollum@aphis.usda.gov

cc

08/09/2010 09:18 PM

Subject: Fwd: bison project

Matt

Take a look at what (b) (6) has put together. Let me know if you see any problems

(b) (6)

Begin forwarded message:

From: (b) (6) @yahoo.com>

Date: August 9, 2010 9:06:33 PM MDT

To: (b) (6) @gmail.com>

Subject: Re: bison project

Hi (b) (6)

Let me know if this works better. I saved it in a compatibility format.

(b) (6)

--- On Mon, 8/9/10, (b) (6) @gmail.com> wrote:

From: (b) (6) @gmail.com>

Subject: Re: bison project

To: (b) (6) @yahoo.com>

Date: Monday, August 9, 2010, 9:01 PM

Hey (b) (6)

The file you sent is in a format I can not open. It has ZIP on the front of the icon

Duane

On Aug 9, 2010, at 5:05 PM, (b) (6) wrote:

(b) (6),

I have attached a write up of the semen freezing and embryo in vitro production procedure that we would use with the bison material. Please let me know if it is sufficient. I can always add more detail.

I will be more than happy to be present at all of the procedures conducted by Matt. I also think that will be best so that I can ensure that the sample(s) aren't accidentally compromised.

I will inquire about having someone there to photograph our work if it must be done while you are gone. I know some people in the community who are amateur photographers and would probably be happy to help.

Please feel free to give Matt my contact information so that he can contact me directly to set up a time to for the semen collections.

All my best,

(b) (6)

--- On Fri, 8/6/10, (b) (6) <(b) (6)@gmail.com> wrote:

From: (b) (6) <(b) (6)@gmail.com>

Subject: bison project

To: (b) (6) <(b) (6)@yahoo.com>

Date: Friday, August 6, 2010, 10:51 AM

Hi (b) (6)

I spoke to Matt McCollum at USDA on the buffalo cow. He thought they could set up to collect the bulls in a morning before the 18th. It sounded like they will dart them to collect the semen. They have done this before to test the semen. He wondered if you could be there when they collect the bulls to be sure the semen is handled properly.

Also could you put a short write together on how we are going to do all this for myself and Matt to have.

While I have a good grasp of the process it would be good to have something wrote down.

I will not be able to be there if this all happens between the August 14 and Sept 1. So could you take several photos of the cow live, collecting the bulls etc. I would like you to be in some of the photos as well. I have a friend who has done a lot of documentaries over the years and we thought it would be nice to document what might be the first Yellowstone bison used in this method of conservation. If the time line was different he would probably come down with me to film it.

Let me know if you have questions

Thanks

(b) (6)

<In Vitro Fertilization trial with reproductive material from Yellowstone Bison.docx>

In Vitro Fertilization trial with reproductive material from Yellowstone Bison

Last modified August 9, 2010 by
Jennifer Barfield, PhD

Collection and cryopreservation of semen

Two bison bulls will be darted and semen collected by electro-ejaculation. Fresh semen will be transferred to the laboratory where Dr. Barfield will freeze the semen according to published procedures (1,2). We have previously frozen epididymal spermatozoa using these methods. These spermatozoa were thawed and used in IVF trials with both cattle and bison oocytes in our lab at Colorado State University and produced viable embryos.

Prior to cryopreservation, the semen will be analyzed for concentration, motility, and morphology. For cryopreservation, the semen will be diluted with a Tris-egg yolk diluent with 6% glycerol to a concentration of 50-200 $\times 10^6$ sperm/mL depending on the concentration of the raw semen sample. Diluted semen will be packaged into 0.5 mL straws and frozen according to the following freezing curve: 22 to 4°C at -0.2°C/min, hold at 4°C for 30 min, 4 to -15°C at -3°C/min, -15°C to -80°C at -10°C/min, hold at -80°C for 10 min, plunge into liquid nitrogen.

Collection of bison oocytes and in vitro production of embryos

The bison cow will be euthanized and the ovaries immediately removed, rinsed and held in saline at room temperature (28-30 °C). Ovaries will be transferred to the laboratory of Dr. George Seidel at the Animal Reproduction and Biotechnology lab at CSU foothills campus. Approximately 4 hours after the ovaries are removed from the cow, oocytes will be aspirated using a vacuum pump system connected to 50 mL conical tubes. Contents of ovarian follicles will be collected in the tubes and then diluted with a buffered holding medium while oocytes are isolated and transferred to a maturation medium. Selected oocytes will be matured in the bovine maturation medium for 23 hours in an incubator with 5%CO₂ and 20% O₂ at 38.5°C. After 23 hours of incubation, oocytes will be fertilized with frozen-thawed bison sperm.

Briefly, sperm will be thawed by submersing the straw of semen in a 37°C water bath for 30 sec. The contents of the straw will then be expelled onto a 45/90% Percoll gradient and centrifuged to separate the spermatozoa from the extended semen. The Percoll and semen will be removed from the tube leaving only the pellet of sperm at the bottom of the tube. Four mL of a buffered holding solution will be added to the pellet and the suspension will be centrifuged once again to rinse the spermatozoa. Following this rinse, spermatozoa will be diluted to 5 $\times 10^6$ /mL with fertilization medium. Approximately 500,000 sperm will be added to a well of 500 μ L fertilization medium containing all of the collected oocytes. Sperm and oocytes will be co-incubated for 18 h in an incubator with 5%CO₂ and 20% O₂ at 38.5°C.

Following the 18 h fertilization period, cumulus cells are removed from the oocytes in a process called vortexing. This procedure involves transferring the oocytes to tubes and vigorously spinning them to strip them of any cells that may be stuck to the outside of the oocytes. The oocytes are then rinsed and put into the first of 2 culture media (CDM-1) and incubated in 5% CO₂, 5% O₂, and 20% N₂ at 38.5°C.

Embryos are removed from CDM-1 approximately 56 hours after the initiation of incubation. Embryos are transferred to warm H-CDM-2 for evaluation. A morphological evaluation is made of the embryos to determine if they have divided. Embryos can be recorded as 1 cell (unfertilized ova), fragmented (unevenly cleaved), 2-3 cells, 4-6 cells, and 8 or more cells. Those recorded as 1 cell and fragmented, will be considered uncleaved and those recorded in the other categories will be considered as cleaved. The percent of cleaved embryos is considered as a measurement of fertilization rate, although the cleavage rate may be higher or lower than the real rate of fertilization. Some oocytes penetrated by sperm do not cleave, while others cleave parthenogenetically or are polyspermic. Uncleaved oocytes and those with only 2-3 cells are discarded. They could be a detrimental factor in the future development of cleaved embryos, assuming that they are dying cells. Cleaved embryos are transferred to 500 μ L warm, equilibrated CDM2 (≤ 35 embryos per well) and incubated at 38.5°C in 5% CO₂, 5% O₂, 90% N₂.

Day 7 (~ 168 h from onset of IVF): At this time, embryos normally have reached the blastocyst stage (early, mature, expanded). An embryo reaching the blastocyst stage at this time is considered as successfully developed; therefore, the percent of blastocysts at this time is considered as the best indicator of the performance of the IVM, IVF and IVC procedures, although quite a few additional blastocysts develop by half a day later, when hatching is starting to occur. Any embryos reaching the blastocyst stage will be cryopreserved by a vitrification method.

References

1. Aurini LC, Whiteside DP, Elkin BT, Thundathil JC. 2009. Recovery and cryopreservation of epididymal sperm of plains bison (*Bison bison bison*) as a model for salvaging the genetics of wood bison (*Bison bison athabasca*). *Reprod Dom Anim* 44, 815-822.
2. Lessard C, Danielson J, Rajapaksha K, Adams GP, McCorkell R. 2009. Banking North American buffalo semen. *Theriogenology* 71, 1112-1119.

From: [Jack C Rhyan](#)
To: [Pauline Nol](#)
Subject: Fw: Bison Study protocol
Date: Friday, January 21, 2011 9:20:00 AM

FYI

----- Forwarded by Jack C Rhyan/CO/APHIS/USDA on 01/21/2011 09:19 AM -----

**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

Jack, I am glad you sat in on QA's presentation of our new protocol template. Unfortunately the new template leads the Bison study into the new Category 4 classification (full protocol). Do you have access to our intranet? If so, you can download the template there. If not I can get it for you. If you can work on reformatting your protocol into the new template, I can help you finish it up.

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

From: [McCollum, Matthew P - APHIS](#)
To: [Nol, Pauline - APHIS](#); [Rhyan, Jack C - APHIS](#)
Subject: FW: Bison to NWRC
Date: Friday, June 19, 2015 12:41:11 PM
Attachments: [ATT00001.txt](#)

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.

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
>
> 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
>
> <1-27 Bison To NWRC 21 Aug 14.pdf>

From: [Nol, Pauline - APHIS](#)
To: [Frey, Rebecca K - APHIS](#)
Subject: FW: Bison to NWRC
Date: Monday, June 22, 2015 9:09:00 AM
Attachments: [ATT00001.txt](#)

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; 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
Subject: Re: Bison to NWRC

Bison received at the VS pens in Fort Collins. See attached.

Thanks all,
Matt

Sent from my iPhone

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Thomas F (APHIS); '"Zaluski, Martin" <MZaluski@mt.gov> "Zaluski, Martin" Martin
Zaluski (MZaluski@mt.gov)'; Frey, Rebecca K - APHIS; 'Pauline Nol'; McCollum,
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> 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>

Wondering if you can fill a blank for us.
For the bison that you are using for the transmission study do you know which bull was used for cow Red03 which aborted in February?

Chris Quance
Microbiologist/Team Leader
National Veterinary Services Laboratories
Diagnostic Bacteriology Laboratory
Mycobacteria and Brucella Section
1920 Dayton Avenue
Ames IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
christine.r.quance@aphis.usda.gov

From: [Rhyon, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Brucella Test Results
Date: Thursday, March 20, 2014 11:00:25 AM

FYI

From: Quance, Christine R - APHIS
Sent: Thursday, March 20, 2014 9:16 AM
To: Clarke, Patrick R. - APHIS
Cc: Rhyon, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: Brucella Test Results

Good Morning,

We isolated *Brucella abortus* biovar 1 from the following tissue submission:

NVSL Acc. 14-004963, Case: B14-0046

Owner: USDA/APHIS/VS/GonaCon Study

Species: Bison

Animal ID: R23

Brucella abortus is a APHIS/CDC Select Agent, and requires reporting to the APHIS/CDC Select Agent Program whenever it is identified.

I need you to reply to this email to acknowledge that you have been informed of this identification, and to answer the following questions:

- 1)** Was there any exposure that occurred during sample collection?
- 2)** Did you retain or send any samples elsewhere?

Thanks!

Chris Quance
Microbiologist
National Veterinary Services Laboratories
Diagnostic Bacteriology Laboratory
Mycobacteria and Brucella Section
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.r.quance@aphis.usda.gov

From: [Rhyon, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: Brucella Test Results
Date: Thursday, March 20, 2014 2:55:20 PM

FYI

From: Frey, Rebecca K - APHIS
Sent: Thursday, March 20, 2014 2:22 PM
To: Rhyon, Jack C - APHIS
Subject: Re: Brucella Test Results

No sure all of them, but Ryan collected lymph nodes. She was caught in the gate, never recovered in January.

Becky
USDA APHIS VS
Sent from my iPhone

On Mar 20, 2014, at 1:36 PM, "Rhyon, Jack C - APHIS" <Jack.C.Rhyon@aphis.usda.gov> wrote:

Becky,
What tissue did we submit on this one?
Jack

From: Quance, Christine R - APHIS
Sent: Thursday, March 20, 2014 9:16 AM
To: Clarke, Patrick R. - APHIS
Cc: Rhyon, Jack C - APHIS; Frey, Rebecca K - APHIS
Subject: Brucella Test Results

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Thanks!

Chris Quance
Microbiologist
National Veterinary Services Laboratories
Diagnostic Bacteriology Laboratory
Mycobacteria and Brucella Section
1920 Dayton Avenue
Ames, IA 50010
Ph: 515-337-7347
Fax: 515-337-7315
Christine.r.quance@aphis.usda.gov

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: calving chart
Date: Friday, June 21, 2013 10:00:14 AM

From: Frey, Rebecca K - APHIS
Sent: Wednesday, June 19, 2013 11:18 AM
To: Rhyan, Jack C - APHIS
Subject: calving chart

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-mummy	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		
29-Apr	Green 14	calf	Neg	Neg		
7-May	Red 13	calf	Pos	Pos		
8-May	Red 25	calf	Pos	Pos		
8-May	Green 08	calf	Neg	Neg		
10-May	Red 22	calf	Pos	Pos		
14-May	Red 07	calf	Susp	Pos		
14-May	Red 08	calf	Pos	Pos		
16-May	Red 20	calf	Pos	Pos		
16-May	Red 18	calf	Pos	Pos		
19-May	Red 30	calf	Pos	Pos		
23-May	Green 17	calf	Neg	Neg		(Rigler)
24-May	Red 26	calf	Pos	Pos		
5-Jun	Green 02	calf	Neg	Neg		(Rigler)
11-Jun	Green 03	calf	Neg	Neg		(Rigler)

This is the entirety of what I know so far. Hope it helps!

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyon, Jack C - APHIS
Sent: Tuesday, June 18, 2013 4:57 PM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: bison origins

I had a conversation with Suelee about the brucella genetics they are getting from our isolates. Pretty interesting and it will get more so as we observe transmission from one animal to another. I wrote a paragraph describing the origin of the animals from which she has obtained isolates recently and I would like you to correct it where it is wrong. I did it pretty much from my memory and we know that isn't very reliable. Her results so far have shown isolates from all animals in the study are identical except for R 21 which has some differences. Also what dates did R 16 and R 21 abort?

Thanks much,
Jack

From: [Frey, Rebecca K - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: calving chart
Date: Monday, June 24, 2013 1:45:28 PM

This is what we know so far as to seroconversions and tissue culture.

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyan, Jack C - APHIS
Sent: Wednesday, June 19, 2013 12:05 PM
To: Frey, Rebecca K - APHIS
Subject: RE: calving chart

Perfect! Thanks Becky and I'm sorry for the barrage of lab results.

This is actually a very cool study. We will learn a lot about bruc in bison that the old field study in the park couldn't tell us. I I can't wait to see if interspecies transmission might occur (:

From: Frey, Rebecca K - APHIS
Sent: Wednesday, June 19, 2013 11:18 AM
To: Rhyan, Jack C - APHIS
Subject: calving chart

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-mummy	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		
29-Apr	Green 14	calf	Neg	Neg		
7-May	Red 13	calf	Pos	Pos		
8-May	Red 25	calf	Pos	Pos		
8-May	Green 08	calf	Neg	Neg		
10-May	Red 22	calf	Pos	Pos		
14-May	Red 07	calf	Susp	Pos		
14-May	Red 08	calf	Pos	Pos		
16-May	Red 20	calf	Pos	Pos		
16-May	Red 18	calf	Pos	Pos		
19-May	Red 30	calf	Pos	Pos		
23-May	Green 17	calf	Neg	Neg		(Rigler)
24-May	Red 26	calf	Pos	Pos		

5-Jun	Green 02	calf	Neg	Neg		(Rigler)
11-Jun	Green 03	calf	Neg	Neg		(Rigler)

This is the entirety of what I know so far. Hope it helps!

Rebecca Frey
Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyon, Jack C - APHIS
Sent: Tuesday, June 18, 2013 4:57 PM
To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS
Subject: bison origins

I had a conversation with Suelee about the brucella genetics they are getting from our isolates. Pretty interesting and it will get more so as we observe transmission from one animal to another. I wrote a paragraph describing the origin of the animals from which she has obtained isolates recently and I would like you to correct it where it is wrong. I did it pretty much from my memory and we know that isn't very reliable. Her results so far have shown isolates from all animals in the study are identical except for R 21 which has some differences. Also what dates did R 16 and R 21 abort?

Thanks much,
Jack

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#)
Subject: FW: Case Report / Owner: None Provided / Ref: 421 / F1532360*Final 1 / Submitter: Rhyan, Jack
Date: Saturday, May 16, 2015 6:31:15 AM
Attachments: [F1532360_Final_1.PDF](#)

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

From: (b) (6)@colostate.edu [mailto:(b) (6)colostate.edu]

Sent: Friday, May 15, 2015 2:22 PM

To: Rhyan, Jack C - APHIS

Subject: Case Report / Owner: None Provided / Ref: 421 / F1532360*Final 1 / Submitter: Rhyan, Jack

Please find attached your reports from the Veterinary Diagnostic Laboratories at Colorado State University.

This email account is not monitored continuously and if you use the reply button it may take a while for us to respond.

If you need immediate assistance please contact the lab at (970) 297-1281

Laboratory Report Final

*This report supersedes all
previous reports for this case*

Case #: F1532360
Referral #: 421
Date Collected:
Date Received: 04/23/2015
Case Coordinator: Dr. Tawfik Aboellail
Owner: None Provided

Email To: jack.c.Rhyan@APHIS.usda.gov
 NWRC/Vet Services
 Dr. Jack Rhyan
 4101 Laporte Ave.
 Fort Collins, CO 80521

**Electronically Signed and Authorized
By:**
 Dr. Tawfik Aboellail
 sent by Cindy Arrieta
 on 5/15/2015 2:21:54PM

Case Contacts

Bill To	NWRC/National Wildlife Research Center	970-266-6140	JACK.C.RHYAN@APHIS.USDA.GOV
Report To	Nol, Pauline	970-266-6126	pauline.nol@aphis.usda.gov
Report To	Bahr, Michelle		michelle.1.bahr@APHIS.usda.gov
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

Specimen Details

ID	Taxonomy	Sex	Age
421	American Bison	Female	

Owner: None Provided

Specimens Received: Blood; Body; Brain Tissue; Tissue Pool;

Clinical History

Bison found dead on morning of 4/23/15 – autolyzed, tissues friable, unclotted blood.

Laboratory Findings/Diagnosis

DIAGNOSIS: Multifocal, acute, mild, ulcerative stomatitis with cheek papillary necrosis.

COMMENTS: PCR against bovine herpes virus 2, malignant catarrhal fever is positive. PCR for anthrax was negative on pooled lung tissue and ear notch. Also, rabies FA testing was negative.

GROSS NECROPSY: A head of a young bison was submitted for necropsy. The palatine mucosa was multifocally ulcerated and there was bilateral focal necrosis of cheek papillae.

HISTOPATHOLOGY: Several sections of brain are examined. No significant histologic lesions were present in these sections of brain from cerebellum, brainstem, mid-brain, and cerebrum.

Tawfik A. Aboellail, BVSc, MVSc, PhD, DACVP
 Dictated: 4/27/15 TAA
 Full report: 5/12/15 TAA Imj

Virology

Rabies FA

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0004	Brain Tissue	24-Apr-2015	Negative

B S L 3

Bacillus anthracis (Anthrax) real-time PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	2	Blood	24-Apr-2015	Negative
421	3	Tissue Pool	24-Apr-2015	Negative Lung and Ear notch pool

M o l e c u l a r D i a g n o s t i c s

Caprine Herpesvirus (CapHV-1) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0005	Tissue Pool	28-Apr-2015	Negative Cheek mucosa, lymph node & lung were pooled for testing.

Ovine Herpesvirus 2 (OHV-2 MCF) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	F1532360-01.0005	Tissue Pool	27-Apr-2015	Positive Cheek mucosa, lung and lymph node were pooled for testing.

N e c r o p s y

Necropsy Wildlife / Exotics Gross Examination Only

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	1	Body	15-May-2015	Complete

End of Report

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Case Report / Owner: None Provided / Ref: PO#REQ17551 / F1532366*Final 1 / Submitter: Rhyan, Jack
Date: Friday, May 15, 2015 5:18:35 PM
Attachments: [F1532366_Final_1.PDF](#)

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

From: (b) (6) @colostate.edu [[mailto:\(b\) \(6\) @colostate.edu](mailto:(b) (6) @colostate.edu)]

Sent: Friday, May 15, 2015 2:12 PM

To: Rhyan, Jack C - APHIS

Subject: Case Report / Owner: None Provided / Ref: PO#REQ17551 / F1532366*Final 1 / Submitter: Rhyan, Jack

Please find attached your reports from the Veterinary Diagnostic Laboratories at Colorado State University.

This email account is not monitored continuously and if you use the reply button it may take a while for us to respond.

If you need immediate assistance please contact the lab at (970) 297-1281

Laboratory Report Final

*This report supersedes all
previous reports for this case*

Case #: F1532366
Referral #: PO#REQ17551
Date Collected: 04/23/2015
Date Received: 04/23/2015
Case Coordinator: Dr. Terry Spraker
Owner: None Provided

Email To: jack.c.Rhyan@APHIS.usda.gov
NWRC/Vet Services
Dr. Jack Rhyan
4101 Laporte Ave.
Fort Collins, CO 80521

**Electronically Signed and Authorized
By:**
Dr. Terry Spraker
sent by Cindy Arrieta
on 5/15/2015 2:11:42PM

Case Contacts

Bill To	NWRC/National Wildlife Research Center	970-266-6140	JACK.C.RHYAN@APHIS.USDA.GOV
Report To	Bahr, Michelle		michelle.1.bahr@APHIS.usda.gov
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

Specimen Details

ID	Taxonomy	Sex	Age
Piglet Found Dead 4/21/15	Porcine	Male	7.0 Weeks
Teddy's Runt Piglet	Porcine	Female	5.0 Weeks

Owner: None Provided

Specimens Received: Feces; Heart Tissue; Liver Tissue; Mesenteric; Tracheobronchial;

Bacteriology

Aerobic & Anaerobic Culture - Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
Piglet Found Dead 4/21/15	2	Heart Tissue	27-Apr-2015	Clostridium species Light growth Final 05/01/2015 E. coli Heavy growth
Piglet Found Dead 4/21/15	3	Liver Tissue	27-Apr-2015	Clostridium perfringens Light growth Clostridium species Light growth Final 05/01/2015 E. coli Heavy growth
Piglet Found Dead 4/21/15	5	Tracheobronchial	27-Apr-2015	Clostridium species Light growth Final 05/01/2015 E. coli Heavy growth

Aerobic Culture - Feces

Animal/Source	Specimen	Specimen Type	Result Date	Results
Teddy's Runt Piglet	1	Feces	27-Apr-2015	Mixed enterics Moderate growth

Owner: None Provided

				No Salmonella Isolated
Piglet Found Dead 4 4/21/15		Mesenteric	27-Apr-2015	Mixed enterics Moderate growth No Salmonella Isolated

Clostridium Fecal Culture Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
Teddy's Runt Piglet 1		Feces	01-May-2015	No Clostridia Isolated
Piglet Found Dead 4 4/21/15		Mesenteric	28-Apr-2015	Clostridium perfringens 2+

End of Report

From: [Rhyan, Jack C - APHIS](#)
To: [Nol, Pauline - APHIS](#); [McCollum, Matthew P - APHIS](#)
Subject: FW: Case Report / Owner: None Provided / Ref: REQ17377 / F1530903*Final 1 / Submitter: Rhyan, Jack
Date: Saturday, May 16, 2015 6:31:29 AM
Attachments: [F1530903_Final_1.PDF](#)

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

From: (b) (6) colostate.edu [[mailto:\(b\) \(6\) colostate.edu](mailto:(b) (6) colostate.edu)]

Sent: Friday, May 15, 2015 2:19 PM

To: Rhyan, Jack C - APHIS

Subject: Case Report / Owner: None Provided / Ref: REQ17377 / F1530903*Final 1 / Submitter: Rhyan, Jack

Please find attached your reports from the Veterinary Diagnostic Laboratories at Colorado State University.

This email account is not monitored continuously and if you use the reply button it may take a while for us to respond.

If you need immediate assistance please contact the lab at (970) 297-1281

Laboratory Report Final

*This report supersedes all
previous reports for this case*

Case #: F1530903
Referral #: REQ17377
Date Collected: 04/19/2015
Date Received: 04/20/2015
Case Coordinator: Dr. Terry Spraker
Owner: None Provided

Email To: jack.c.Rhyan@APHIS.usda.gov
NWRC/Vet Services
Dr. Jack Rhyan
4101 Laporte Ave.
Fort Collins, CO 80521

**Electronically Signed and Authorized
By:**
Dr. Terry Spraker
sent by Cindy Arrieta
on 5/15/2015 2:18:08PM

Case Contacts

Bill To	NWRC/National Wildlife Research Center	970-266-6140	JACK.C.RHYAN@APHIS.USDA.GOV
Report To	Bahr, Michelle		michelle.1.bahr@APHIS.usda.gov
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

Specimen Details

ID	Taxonomy	Sex	Age
3G02	American Bison	Male	2.0 Years

Owner: None Provided

Specimens Received: Abscess Material, Jaw; Body; Brain Tissue; L Node; Lung Tissue;

Clinical History

This animal was found dead on 4/19/15. There were multifocal abscesses in the head with lymph node involvement. The animal was submitted for rabies and, if rabies was negative, examination or culture of lymph nodes and examination of lung for MCF.

Laboratory Findings/Diagnosis

DIAGNOSIS: Head/skin/lymph nodes: Multiple abscesses with intralesional bacteria.

COMMENTS: The primary lesions found in the head of this bison were multiple abscesses on the lower jaw and adjacent lymph nodes with intralesional bacteria. Evidence of MCF was not found histologically and tests for rabies were negative.

HISTOPATHOLOGY:

Slide 1.

Skin: This slide contains subcutaneous tissue, skeletal muscle, lymph nodes, and multiple abscesses. These abscesses are characterized by circumscribed focus filled with degenerating neutrophils with a mixture of macrophages and lymphocytes. Bacterial colonies are found surrounded by Splendore-Hoeppli material.

Slide 2.

Lung: This section of lung has moderate edema, but no evidence of inflammation.

Slide 3.

Skin, lower jaw: This section of skin does contain multiple abscesses with intralesional bacteria. These abscesses are surrounded by a thin layer of fibrosis associated with lymphocytes, neutrophils, and macrophages. Colonies of intralesional bacteria are observed within these abscesses surrounded by Splendore-Hoeppli formation.

Slide 4.

Owner: None Provided

Pituitary gland with cerebral retes: The pituitary gland is normal. There is no evidence of vasculitis within this cerebral rete, suggesting this animal does not have MCF.

Slide 5.

Brain: Multiple sections of brain, including the caudate nucleus, corpus striatum, thalamus, hippocampus, spinal cord, and cerebrum are examined: All are within normal limits.

Terry R. Spraker, DVM, PhD, DACVP

Prelim: 4/25/15 TRS

Full report: 5/14/15 Imj

Bacteriology**Aerobic & Anaerobic Culture - Food Animal**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	4	L Node	22-Apr-2015	Bacillus species Light growth E. coli Light growth No Anaerobes Isolated Final 04/27/2015 Proteus mirabilis Light growth

Aerobic Culture Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	F1530903-01.0005	Abscess Material, Jaw	23-Apr-2015	Acinetobacter species Moderate growth Bacillus species Moderate growth Pasteurella pneumotropica Moderate growth Final 4/27/15

Virology**Rabies FA**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	1	Brain Tissue	21-Apr-2015	Negative

Molecular Diagnostics**Caprine Herpesvirus (CapHV-1) - PCR**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	23-Apr-2015	Negative

Ovine Herpesvirus 2 (OHV-2 MCF) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	23-Apr-2015	Negative

End of Report

From: [Rhyan, Jack C - APHIS](#)
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?
Date: Tuesday, February 12, 2013 1:31:30 PM

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

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Cc: Frey, Rebecca K - APHIS

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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

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Cc: Nol, Pauline - APHIS; McCollum, Matthew P - APHIS

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Before noon on 20th, or before 2:30 on 19th

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USDA APHIS VS
Sent from my iPhone

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P. Ryan Clarke, DVM, MPH
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Wildlife Disease Specialist

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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

" 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.

P. Ryan Clarke, DVM, MPH

Regional Epidemiologist-GYA

USDA-APHIS-VS-WR

406-388-5162

From: Rhyan, Jack C - APHIS

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

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

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.

Jack

From: Frey, Rebecca K - APHIS

Sent: Monday, February 11, 2013 3:50 PM

To: Rhyan, Jack C - APHIS; Clarke, Patrick R. - APHIS

Cc: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

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.

Rebecca Frey

Wildlife Disease Specialist
USDA APHIS Veterinary Services
Montana
406-333-4425

From: Rhyan, Jack C - APHIS

Sent: Monday, February 11, 2013 1:51 PM

To: Frey, Rebecca K - APHIS; Clarke, Patrick R. - APHIS

Cc: McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

Subject: RE: Conference call about GonaCon (2nd rendition) ? Elk study at Brogan's?

Either would work for me. Also, Rick has been asking me to get the Coop agreement in place as they have about 200 bison in the Gardiner basin and he thinks the next 2-3 weeks will be prime for capture. I sent it to Mark and asked him to call me. Are ya'll okay with it if I go ahead and try to get signatures on it and get it back to Rick? I read it over and I think its benign.

Jack

From: Frey, Rebecca K - APHIS

Sent: Monday, February 11, 2013 1:36 PM

To: Clarke, Patrick R. - APHIS

Cc: Rhyan, Jack C - APHIS; McCollum, Matthew P - APHIS; Nol, Pauline - APHIS

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

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
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406-388-5162

From: [Nol, Pauline - APHIS](#)
To: [Rhyan, Jack C - APHIS](#); [Frey, Rebecca K - APHIS](#); [Clarke, Patrick R. - APHIS](#)
Subject: FW: conference call
Date: Monday, April 07, 2014 3:57:00 PM

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
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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

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
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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
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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

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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

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