



# MVDL

## MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59771  
1911 West Lincoln Street Bozeman, MT 59718  
Website: [www.liv.mt.gov/lab](http://www.liv.mt.gov/lab)

Phone: (406) 994-4885  
Fax: (406) 994-6344  
Email: [livdiagnosticlab@mt.gov](mailto: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

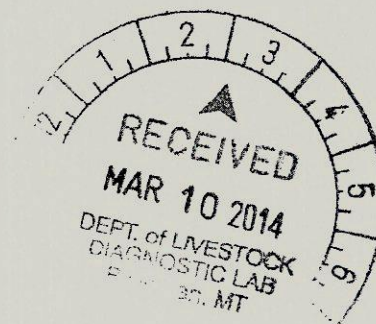
**This is not a billable case.**

**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](mailto: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

*L. H. Hinkle DVM*

Designated Brucellosis Epidemiologist



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

030514



Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison
Study Director:	Jack Rhyan
:	



## REGULATORY CONSIDERATIONS

Permits					
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <table style="width: 100%; border: none;"> <tr> <td style="border: none; width: 50%;">Permit(s) description</td> <td style="border: none; width: 25%;">Number</td> <td style="border: none; width: 25%;">Date</td> </tr> </table>	Permit(s) description	Number	Date
Permit(s) description	Number	Date			

## DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
  - ☒ *Manuscript/review article collaboration*
  - ☐ *Training program requiring the use of animals*
  - ☒ *Data analysis, interpretation and reporting*
  - ☒ *Other: \_\_\_\_\_ Live animal work \_\_\_\_\_*

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

## STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator



Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Attending veterinarian
Jason Lombard	USDA, APHIS, VS	Investigator
Jenny Powers	National Park Service	Investigator
Rick Wallen	National Park Service	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

## 2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 <sup>th</sup> 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<sup>TM</sup> 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.

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:

5/16/11

Concur:

IACUC Chair

Date



## Necropsy 421

Thursday, April 23, 2015

Bison, Female, 3 yr

Animal found dead in second pen from the western side of inner facility at APHI/APHIS WRF. This animal was transported to Fort Collins in January, 2015 from the Bison Quarantine Facility in Corwin Springs, MT. This was an excess animal from a Gonacon study. This animal is *Brucella* seropositive.

Animal was in fair body condition.

On necropsy, tissues were noted to have marked autolysis. All tissues were dark and friable. Extensive green discoloration of tissue surfaces noted.

Copious unclotted blood in thoracic cavity and abdominal cavity

GI tract: very loose stool.

Lungs: Green surface. Floated in formalin.

Heart: Enlarged, flabby

Head: not observed. Submitted to Colorado State University for diagnostics

Collected: prescapular Inn, popliteal In, lung, spleen, liver, kidney, ruminal Inn, iliac Inn, mesenteric Inn, colon with feces, ileum, ileocecolic Inn, heart.

Submitted: head for rabies FA, lung/prescapular Inn for OHV-2 and CHV-1 PCR; blood for *Bacillus anthracis* PCR.

Colorado State University results:

Multifocal, acute, mild ulcerative stomatitis with cheek papillary necrosis

Rabies FA negative

OHV-2 positive on lung/In/cheek lesion pool

CHV-1 PCR negative

*Bacillus anthracis* negative on blood and ear notch/lung pool



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

**Report of:**  
Dr. Tawfik Aboellail  
sent by Christina Weller  
on 4/27/2015 5:11:55PM

#### 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
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

#### Specimen Details

<b>ID</b>	<b>Taxonomy</b>	<b>Sex</b>	<b>Age</b>
421	American Bison	Female	

**Owner:** None Provided

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

#### Laboratory Findings/Diagnosis

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

Test for malignant cattarrhal fever is pending. MCF is the primary rule-out.  
Real-time PCR for anthrax is negative. Rabies testing is also pending.

#### Virology

##### Rabies FA

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

#### BSL 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

#### Molecular Diagnostics



Owner: None Provided

**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		Billing Pending

End of Report

Darting of R52 Bison Cow (with calf) with NalMed

May 6, 2015

R52 is Brucella positive

Gave birth to live calf discovered morning of May 5, 2015

Cow samples: serum, hep whole blood, vaginal swab, placenta, milk (all 4 quarters)

Cow received 8 ml ivermectin sq

8-way vaccine 2ml sq

Python ear tags x 2

Calf is male in good condition

Ear tag 47

Calf samples taken: conjunctival swab, rectal swab, serum, hep whole blood

Betadine Tx to navel

Calf received 8 way vaccine 2 ml sq

0.5ml ivermectin sq

Rotavirus/coronavirus vaccine oral

IBR vaccine sq



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

**Report of:**  
 Dr. Tawfik Aboellail  
 sent by Christina Weller  
 on 4/27/2015 5:11:55PM

#### 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
Submitter	Rhyan, Jack	970-266-6140	jack.c.Rhyan@APHIS.usda.gov

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<b>ID</b>	<b>Taxonomy</b>	<b>Sex</b>	<b>Age</b>
421	American Bison	Female	

**Owner:** None Provided

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#### Virology

##### Rabies FA

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

#### BSL 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

#### Molecular Diagnostics

Owner: None Provided

**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		Billing Pending

End of Report



## Necropsy 421

Thursday, April 23, 2015

Bison, Female, 3 yr

Animal found dead in second pen from the western side of inner facility at APHI/APHIS WRF. This animal was transported to Fort Collins in January, 2015 from the Bison Quarantine Facility in Corwin Springs, MT. This was an excess animal from a Gonacon study. This animal is *Brucella* seropositive.

Animal was in fair body condition.

On necropsy, tissues were noted to have marked autolysis. All tissues were dark and friable. Extensive green discoloration of tissue surfaces noted.

Copious unclotted blood in thoracic cavity and abdominal cavity

GI tract: very loose stool.

Lungs: Green surface. Floated in formalin.

Heart: Enlarged, flabby

Head: not observed. Submitted to Colorado State University for diagnostics

Collected: prescapular Inn, popliteal In, lung, spleen, liver, kidney, ruminal Inn, iliac Inn, mesenteric Inn, colon with feces, ileum, ileocecolic Inn, heart.

Submitted: head for rabies FA, lung/prescapular Inn for OHV-2 and CHV-1 PCR; blood for *Bacillus anthracis* PCR.

Colorado State University results:

Multifocal, acute, mild ulcerative stomatitis with cheek papillary necrosis

Rabies FA negative

OHV-2 positive on lung/In/cheek lesion pool

CHV-1 PCR negative

*Bacillus anthracis* negative on blood and ear notch/lung pool

Necropsy 3G02

Sunday, April 19, 2015

Bison, Male, 2 yr

Animal found dead in western most pen of inner facility at APHI/APHIS WRF. This animal was transported to Fort Collins in January, 2014 from the Bison Quarantine Facility in Corwin Springs, MT. This was an excess animal from a Gonacon study.

Animal was lying partially under the panels on the southwest aspect of the paddock. Animal presented laterally recumbent on the right side. Extensive hair loss was noted on the left side. Exposed skin was dry and leathery. Animal was in fair to poor body condition.

On necropsy, tissues were noted to have mild autolysis.

GI tract: WNL

Lungs: WNL

Heart: WNL

Head: Marked enlargement of medial retropharyngeal lymph nodes, parotid lymph nodes, submandibular lymph nodes. Numerous caseous abscesses found in lymph nodes as well as in subcutaneous tissues of lateral aspects of the head.

Collected medial retropharyngeal Inn, submandibular Inn, parotid Inn, prescapular Inn, iliac Inn, lung, spleen, liver, mesenteric Inn, heart.

Submitted: submandibular lymph node for aerobic and anaerobic culture, head for rabies FA, lung for OHV-2 and CHV-1 PCR.

Colorado State University results:

Rabies FA negative

OHV-2 and CHV-2 PCR negative

Culture:

Bacillus species

Light growth

E. coli

Light growth

No Anaerobes Isolated

Final 04/27/2015

Acinetobacter species

Moderate growth

Bacillus species

Moderate growth

Pasteurella pneumotropica

Moderate growth Final 4/27/15 Proteus mirabilis

Light growth



Necropsy 3G02

Sunday, April 19, 2015

Bison, Male, 2 yr

Animal found dead in western most pen of inner facility at APHI/APHIS WRF. This animal was transported to Fort Collins in January, 2014 from the Bison Quarantine Facility in Corwin Springs, MT. This was an excess animal from a Gonacon study.

Animal was lying partially under the panels on the southwest aspect of the paddock. Animal presented laterally recumbent on the right side. Extensive hair loss was noted on the left side. Exposed skin was dry and leathery. Animal was in fair to poor body condition.

On necropsy, tissues were noted to have mild autolysis.

GI tract: WNL

Lungs: WNL

Heart: WNL

Head: Marked enlargement of medial retropharyngeal lymph nodes, parotid lymph nodes, submandibular lymph nodes. Numerous caseous abscesses found in lymph nodes as well as in subcutaneous tissues of lateral aspects of the head.

Collected medial retropharyngeal Inn, submandibular Inn, parotid Inn, prescapular Inn, iliac Inn, lung, spleen, liver, mesenteric Inn, heart.

Submitted: submandibular lymph node for aerobic and anaerobic culture, head for rabies FA, lung for OHV-2 and CHV-1 PCR.

Colorado State University results:

Rabies FA negative

OHV-2 and CHV-2 PCR negative

Culture:

Bacillus species

Light growth

E. coli

Light growth

No Anaerobes Isolated

Final 04/27/2015

Acinetobacter species

Moderate growth

Bacillus species

Moderate growth

Pasteurella pneumotropica

Moderate growth Final 4/27/15 Proteus mirabilis

Light growth

Necropsy 3G02

Sunday, April 19, 2015

Bison, Male, 2 yr

Animal found dead in western most pen of inner facility at APHI/APHIS WRF. This animal was transported to Fort Collins in January, 2014 from the Bison Quarantine Facility in Corwin Springs, MT. This was an excess animal from a Gonacon study.

Animal was lying partially under the panels on the southwest aspect of the paddock. Animal presented laterally recumbent on the right side. Extensive hair loss was noted on the left side. Exposed skin was dry and leathery. Animal was in fair to poor body condition.

On necropsy, tissues were noted to have mild autolysis.

GI tract: WNL

Lungs: WNL

Heart: WNL

Head: Marked enlargement of medial retropharyngeal lymph nodes, parotid lymph nodes, submandibular lymph nodes. Numerous caseous abscesses found in lymph nodes as well as in subcutaneous tissues of lateral aspects of the head.

Collected medial retropharyngeal Inn, submandibular Inn, parotid Inn, prescapular Inn, iliac Inn, lung, spleen, liver, mesenteric Inn, heart.

Submitted: submandibular lymph node for aerobic and anaerobic culture, head for rabies FA, lung for OHV-2 and CHV-1 PCR.

Colorado State University results:

Rabies FA negative

OHV-2 and CHV-2 PCR negative

Culture:

Bacillus species

Light growth

E. coli

Light growth

No Anaerobes Isolated

Final 04/27/2015

Acinetobacter species

Moderate growth

Bacillus species

Moderate growth

Pasteurella pneumotropica

Moderate growth Final 4/27/15 Proteus mirabilis

Light growth



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

**Report of:**  
Dr. Terry Spraker  
sent by Denise Bolte  
on 4/27/2015 2:12:04PM

#### Case Contacts

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

#### Specimen Details

<b>ID</b>	<b>Taxonomy</b>	<b>Sex</b>	<b>Age</b>
3G02	American Bison	Male	2.0 Years

**Owner:** None Provided

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

#### Laboratory Findings/Diagnosis

Gross finding

Head0Bison

1. Multiple abscesses on lower jaw and adjacent lymph nodes

Histopathology

1. Skin/lymph nodes, multiple abscesses with intralesional bacteria

#### Case Summary

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

#### 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

Owner: None Provided

**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