



## NATIONAL ASSEMBLY of STATE ANIMAL HEALTH OFFICIALS

4221 Mitchell Ave  
Saint Joseph, MO 64507  
Ph: 816-671-1144 Fax: 816-671-1201  
E-Mail: [usaha@usaha.org](mailto:usaha@usaha.org)  
Web Site: [www.usaha.org](http://www.usaha.org)

<b>President</b> <b>Susan Keller DVM</b> 600 E Blvd. Dept 602 Bismarck, ND 58505 701-328-2655 <a href="mailto:skeller@nd.gov">skeller@nd.gov</a>	<b>Vice President</b> <b>Scott Marshall DVM</b> 235 Promenade Street Providence, RI 02908 401-222-2781 <a href="mailto:scott.marshall@dem.ri.gov">scott.marshall@dem.ri.gov</a>	<b>Treasurer</b> <b>Dustin Oedekoven DVM</b> 411 South Fort Street Pierre, SD 57501 605-773-3321 <a href="mailto:dustin.oedekoven@state.sd.us">dustin.oedekoven@state.sd.us</a>	<b>Secretary</b> <b>Tony Frazier DVM</b> 1445 Federal Drive Montgomery, AL 36107 334-240-7253 <a href="mailto:tony.frazier@agi.alabama.gov">tony.frazier@agi.alabama.gov</a>
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July 14, 2017

**To:** The Honorable Sonny Perdue, U.S. Secretary of Agriculture  
**From:** The National Assembly of State Animal Health Officials  
**Subject:** Decommissioning field studies on *Brucella abortus*

The National Assembly of State Animal Health Officials (NASAHO or the National Assembly) is an organization composed entirely of the state and territorial animal health officials of the United States. Our mission is to work collectively to safeguard public and animal health as well as the food supply. We accomplish this by working with federal, state, and industry partners to develop science based policies to address issues that affect public and animal health, public safety, and commerce. We strive to use the best available science to formulate our positions and to reach consensus among all members whenever possible.

The National Assembly is uniquely qualified to assess the impact of animal health threats in our individual states and territories, as well as how those threats will affect our nation. While brucellosis is an expanding zoonotic disease in the Greater Yellowstone Area, the National Assembly is deeply troubled by USDA's (United States Department of Agriculture) decision to decommission critical brucellosis research taking place in Montana and Colorado. Findings from prior research efforts have directly affected decisions relating to management of brucellosis including needed separation in time between the use of lands by infected wildlife and subsequent use by cattle, potential for remote vaccination of wildlife, and most recently, the ability of bull bison to transmit brucellosis. One ongoing study examines transmission of brucellosis from bison treated with contraceptive agents. Non-surgical reproductive control via contraception has the potential to affect not only population growth, but also spread of brucellosis within and from a wildlife population.

The timing of USDA's announcement to decommission brucellosis field studies strikingly conflicts with the recent completion of the National Academy of Sciences (NAS) report, Revisiting Brucellosis in the Greater Yellowstone Area, which concluded that brucellosis is **spreading in wildlife**, and the field **needs more research on elk and bison rather than less**. The NAS report, 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."

Once decommissioned, field research on brucella will not be resumed in the foreseeable future because of high start-up costs, loss of invaluable expertise, and competing interests for limited funding. The increase in prevalence and geographic expansion of brucellosis has been well documented over the last decade. The decision to decommission brucellosis studies puts states that share the geographical range of elk in the Western United States at increased risk.

Also of concern is that the void in research left by APHIS-VS will be inadequately filled by other entities that have previously demonstrated a strong interest in expanding the range of wildlife in the name of conservation, with little regard for the impact of disease to domestic livestock. These entities will have drastically different priorities than to protect agriculture.

The National Assembly understands the role of the Select Agent Program to regulate the use of agents and organisms that may pose a threat to the United States. USDA has indicated that the current field research efforts on brucellosis must be discontinued to ensure compliance with Select Agent regulations, and yet CFR §73.4 (Title 42, Chapter I, Subchapter F), states that, "any overlap select agent or toxin that is in its naturally occurring environment provided that the select agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source," is exempt from the regulations. Studies on captive elk and bison in Montana qualify for this exclusion.

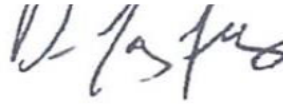
Further, CFR §121.5 (Title 9, Chapter I, Subchapter E, Part 121) allows the APHIS Administrator to grant, "a specific exemption upon a showing of **good cause** and upon his or her determination that such exemption is consistent with protecting animal health or animal products." It is apparent that 'good cause' exists sufficient to grant an exemption to continue valuable research on *B. abortus* with the goal of reducing further spread in the wildlife population and safeguarding our nations livestock herds. It is imperative that we maintain our research capacity.

The National Assembly strongly requests that USDA APHIS continue critical field research on brucellosis based on the expanding range of the disease, lack of alternative study efforts, legal exclusion in CFR §73.4, and ability to grant exemptions through CFR §121.5. The Nation has recognized the strong need for agricultural research through the formation of the USDA-ARS (Agricultural Research Service) which should be involved in ongoing efforts.



Thank you for your consideration.

Submitted on behalf of the National Assembly,



AL State Veterinarian      Tony Frazier DVM, AL State Veterinarian  
Secretary



SD State Veterinarian      Dustin Oedekoven DVM, SD State Veterinarian  
Treasurer

ack.A.Shere@aphis.usda.gov  
e.L.Dick@aphis.usda.gov



# National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

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

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

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FINAL REPORT

## Laboratory Test Report

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

### Owner

APHIS-BQF-GonaCon Study  
Corvis Springs, MT

### Animal Location

Park County MT

### Submitter - 1961

Dr. Patrick Ryan. Clarke  
USDA, APHIS, VS

### Accession Number:

17-008206

### Date Collected:

### Date Received:

03/14/2017

### Date Completed:

### Collected By:

08/10/2017

R. Clarke, et al

### Purpose:

General Diagnostic

### Referral Number:

This is not a billable case.

(b) (6)

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

Sample: Animal ID: Gr 30 Brucella Case Number: B17-0109 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

#### Exudate / Exudate- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

#### Swab / Swab- Vaginal

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

#### Milk / Milk

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

#### Feces / Feces

Brucella Isolation Result

No Isolation Made

**Sample:** Animal ID: Gr 11 **Brucella Case Number:** B17-0110 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

Individual specimen results are listed below:

**Exudate / Exudate- Vaginal**

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

**Exudate / Exudate- Vaginal**

Brucella Isolation Result

Suspect

**Swab / Swab- Vaginal**

Brucella Isolation Result

Isolate Determined

Brucella Identification Result

Brucella abortus

**Milk / Milk**

Brucella Isolation Result

No Isolation Made

**Feces / Feces**

Brucella Isolation Result

No Isolation Made

**Sample:** Animal ID: Red 34 **Brucella Case Number:** B17-0111 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

**Swab / Swab- Vaginal**

Brucella Isolation Result

No Isolation Made

**Milk / Milk**

Brucella Isolation Result

No Isolation Made

**Feces / Feces**

Brucella Isolation Result

Contaminated

**Sample:** Animal ID: Green 18 **Brucella Case Number:** B17-0112 **Specimen Type:** Tissue **Species:** Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

**Exudate / Exudate- Vaginal**

Brucella Isolation Result

No Isolation Made

**Swab / Swab- Vaginal****Swab / Milk**

Brucella Isolation Result

No Isolation Made

**WGS Genotyping Report:**

**The isolates, B17-0109\_17BA\_MT-067\_BI-GonaCon-Gr 30 and B17-0110\_17BA\_MT-067\_BI-GonaCon-Gr 11, share a SNP profile with several GonaCon project bison from 2016.**

**The attached appendix shows a low resolution tree indicating the relationships of these isolates to others throughout the United States, a high resolution tree of the most related isolates, and a table showing the SNP calls for a portion of the group. The isolates of interest are in red font.**

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

**Help Us Help You**

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

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



United States Department of Agriculture

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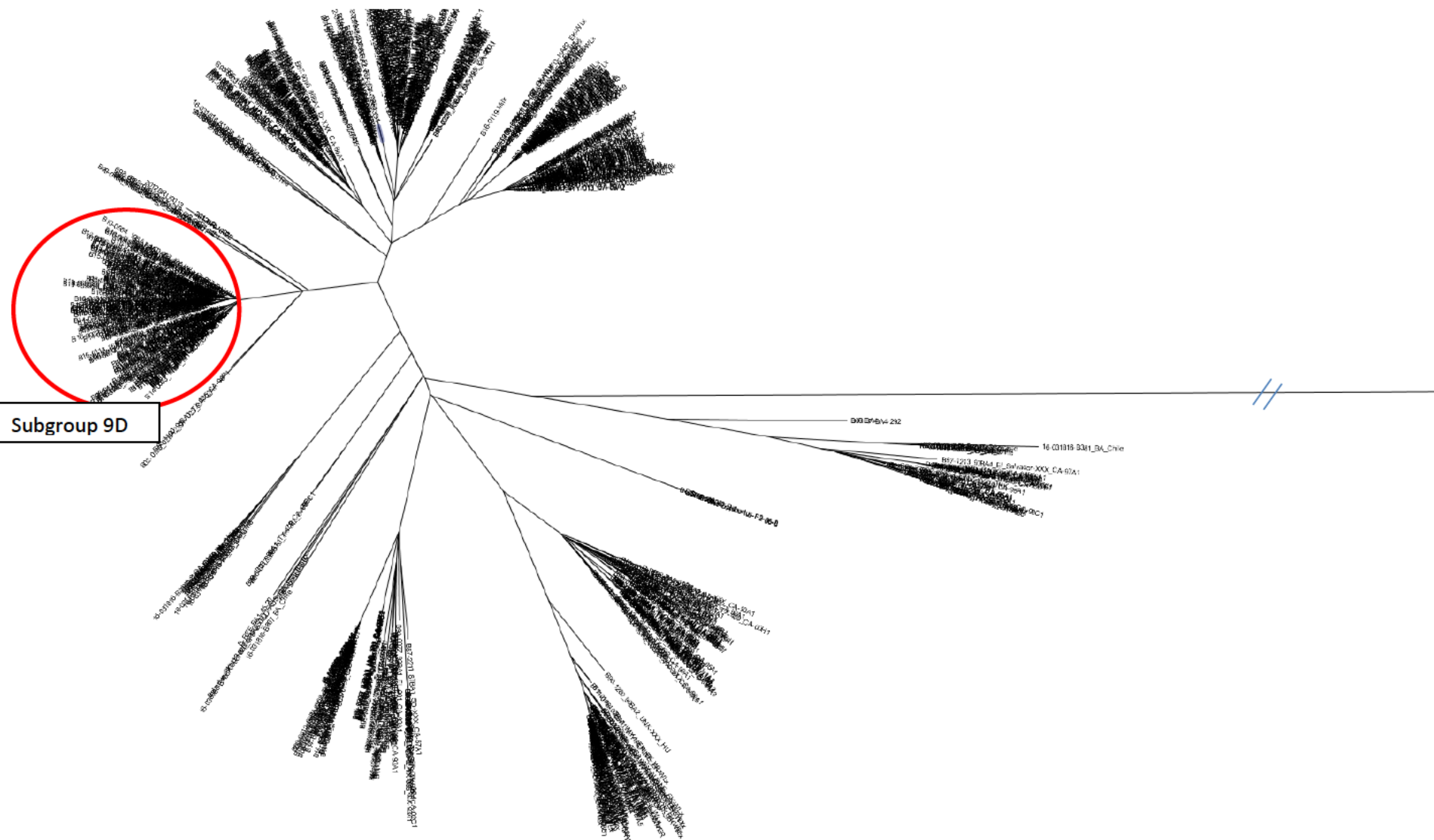
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## APPENDIX – GENOTYPING REPORT

Date: 8/10/2017

Accession: 17-008206

**Figure 1. Low resolution phylogenetic tree detailing the genetic relationship of *Brucella* isolates (n=709) from the NVSL database. The red circle contains the isolates of interest (none are shown at this resolution).**





United States Department of Agriculture

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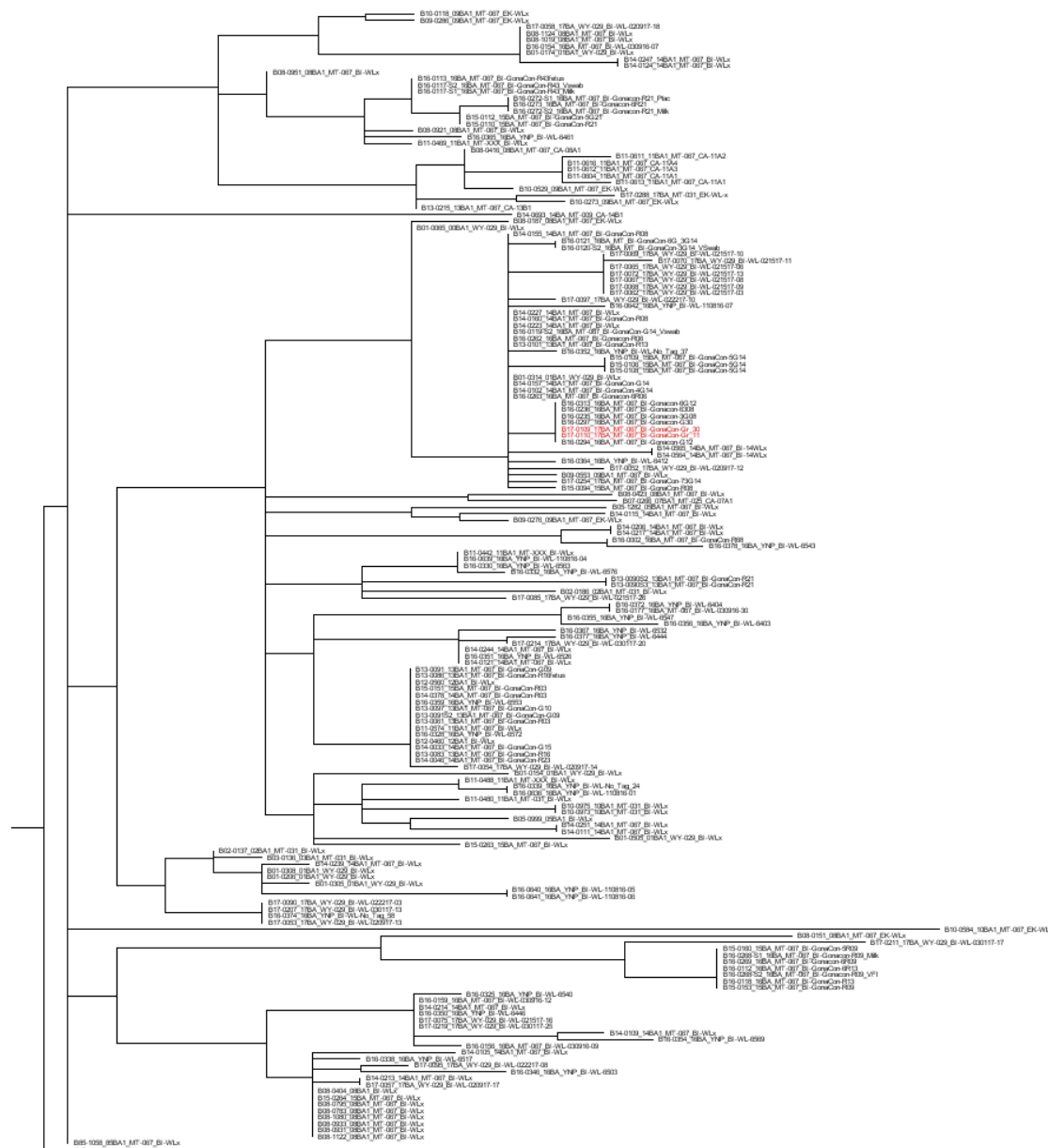
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## APPENDIX – GENOTYPING REPORT

Date: 8/10/2017

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Figure 2. High resolution phylogenetic tree for the isolates circled in Figure 1. The red font indicates the isolates of interest.





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## APPENDIX – GENOTYPING REPORT

Date: 8/10/2017

Accession: 17-008206

**change back to the reference call.**

[illegible]



INTERGOVERNMENTAL AGREEMENT  
FOR GRAZING OF BISON  
AT SOAPSTONE PRAIRIE AND RESERVOIR RIDGE NATURAL AREAS  
AND RED MOUNTAIN OPEN SPACE

THIS INTERGOVERNMENTAL AGREEMENT ("Agreement"), dated \_\_\_\_\_, 2014, is entered into by and between THE CITY OF FORT COLLINS, COLORADO, a municipal corporation ("City"), LARIMER COUNTY, COLORADO ("County"), THE BOARD OF GOVERNORS OF THE COLORADO STATE UNIVERSITY SYSTEM ACTING BY AND THROUGH COLORADO STATE UNIVERSITY, ON BEHALF OF THE ANIMAL REPRODUCTION AND BIOTECHNOLOGY LAB ("CSU"), and THE UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES ("APHIS"), and will be effective on the date last signed below ("Effective Date"). The City, County, CSU and APHIS are referred to herein individually as a "Party" and collectively as the "Parties."

RECITALS

A. Pursuant to a separate agreement between them, CSU and APHIS are collaborating on a program to investigate the use of assisted reproductive techniques as a brucellosis mitigation strategy for North American Bison (the "Project").

B. The majority of the Project work will be conducted at CSU facilities in Fort Collins, and the animals involved will be owned by CSU.

C. The City and County are the owners of certain properties, situated in the County of Larimer, State of Colorado, commonly known as the Soapstone Prairie Natural Area and the Reservoir Ridge Natural Area (City owned), and Red Mountain Open Space (County owned) (together, the "Properties"), and the City and County are willing to make portions of these properties available as grazing land for bison involved in the Project.

D. Bison are listed in the Soapstone Management Plan as a species that the City's Natural Areas Department (NAD) would like to reintroduce to Soapstone. The Project would also benefit the City by providing native grazing animals for grassland management and creating a unique wildlife viewing opportunity for the public.

E. CSU will collaborate with the City and County to conduct surveys that evaluate the impact that bison have on grassland health. This will include non-invasive research conducted by graduate students from CSU, and will also provide opportunities for citizen volunteers to be involved in the Project.

F. Use of the Properties for the Project will also benefit the public by supporting important work in bison genetics, disease mitigation and management, and the implementation of new techniques in the reproductive sciences to help conserve an iconic grassland species.



NOW, THEREFORE, the parties agree as follows:

1. Term and Termination. The term of this Agreement will begin on the Effective Date and continue until termination of the Project. Any Party may also terminate this Agreement at any time on no less than six (6) months advance written notice to the other Parties.

2. Funding.

(a) CSU, APHIS, the City and the County will collaborate to develop funding sources ("Project Funds") to pay for the construction, maintenance and repair of bison fencing, bison handling facilities, water development, and other necessary grazing and grazing management infrastructure, and herd maintenance needs on the Properties, including but not limited to veterinary care and supplemental feed. Funding provided by the City and County will not exceed the amount of funding provided by other partners in the project and will only be used for expenses on the Properties. The City and County are not obligated to provide funding for the Project.

(b) Non-City or County Project Funds will be held and administered by CSU or CSU's designee, and possibly through the CSU Foundation, which can accept tax-deductible donations to be made to the Project.

(c) The City has agreed to provide and install bison fencing on the Properties at a value of up to Forty Thousand Dollars (\$40,000) in matching funds for initiation of the Project on the Properties.

3. Construction of Improvements.

(a) While the initial bison fencing will be installed by the City, other parties may want to place other improvements on the Properties from time to time. No improvements may be placed on any of the Properties by CSU or APHIS without the prior express written approval of the owner of such Property. The City and County will review and approve the location and design of any improvements proposed to be constructed on their respective Properties for the Project, including bison fencing and other grazing infrastructure. Any proposed improvements must be submitted to the owner of the property for its review at least sixty (60) days in advance of the proposed installation. The City and County will, in their reasonable sole discretion, approve or deny any requests for improvements. If a request is denied, the City and/or County will provide CSU and APHIS with a written response with the reason for the denial of approval. The construction of all such improvements on City-owned Properties may need to be contracted through the City's purchasing process, or as otherwise approved by the City's Director of Purchasing and Risk Management.

(b) The City and/or County agree to install signage at the entrance to the Properties that are being used for bison grazing with appropriate information about the Project and bison safety. The Parties shall confer on the type and nature of all such signage.

4. City and County Rights and Responsibilities.

(a) The City and County will provide a portion of the Properties for year-round grazing purposes for the Project, and hereby grant to CSU, APHIS and to each other a license to enter on their respective Properties for the purpose of carrying out the obligations of the parties under this Agreement.

(b) On or about March 1 of each year, commencing in 2015, the City, County and CSU will collaboratively develop an annual written "Grazing Plan" for the Properties, which will include stocking rates, animal unit months ("AUMs") for each pasture, and grazing rotation plans. If the City, County and CSU have not agreed upon a Grazing Plan by May 1 of each year, the City and County will determine the Grazing Plan for that year and provide CSU and APHIS with a written copy of the Grazing Plan..

(c) The parties acknowledge that the City and County currently have cattle grazing programs on Soapstone and Red Mountain and that they intend to maintain those programs. Further, the City may initiate a grazing program on Reservoir Ridge and may, in its sole discretion include the property in this Agreement.

(d) Notwithstanding paragraph 4(b) above, if the City or County determines that the grazing conditions on its respective Property warrant it, whether such conditions are caused by drought, pestilence, insect infestation or any other circumstance beyond the City and County's control, the City or County may reduce the agreed-upon number of AUMs per year on its Property to that number it determines is appropriate under the then-existing grazing conditions. If favorable grazing conditions exist, and the City or County determines that conservation objectives will not be negatively impacted by additional grazing, the City or County may increase the numbers of AUMs per year on its property to a number it determines is appropriate under the then-existing grazing conditions.

(e) In addition to grazing land, the City and County (or their designees) will coordinate with each other to provide the following on the Properties:

- (1) Access to existing water sources for livestock;
- (2) Grazing management, including monitoring of forage use, water availability and range conditions to determine if changes are needed, and reporting any necessary actions to Jennifer Barfield (910-354-8061 or [Jennifer.Barfield@colostate.edu](mailto:Jennifer.Barfield@colostate.edu)) at CSU;
- (3) Periodic herd monitoring including reporting any problems or changes to CSU (contact: Jennifer Barfield or main ARBL office at 970-491-3456); and
- (4) Periodic inspection, maintenance and repair of fencing, water developments, and other grazing infrastructure during normal working hours.



(f) The City and County agree to allow research by CSU faculty and students on the Properties with the approval of the City and/or County. A written summary of the proposed research project must be submitted to the City and/or County for their review and approval at least 30 days prior to the initiation of the study.

5. CSU Rights and Responsibilities.

(a) All bison placed on the Properties will be owned by, and be the responsibility of, CSU. CSU will provide veterinary care, reproductive services, veterinary supplies, and supplemental feed for the animals as needed. All supplemental feeding will be conducted in predetermined locations on the Properties approved in advance in writing as part of the Grazing Plan, and all hay shall be certified "weed free" by a certifying body acceptable to the City and County in their sole discretion. CSU will monitor the animals on a regular basis and will respond promptly to any reports from the City or County of sick or injured animals, escaped animals, or needed changes in stocking or rotation dates based on changed conditions on the Properties. CSU will be responsible for gathering and/capturing any escaped bison.

(b) CSU will administer the Project Funds (see section 2a and 2b) to reimburse reasonable costs for labor and materials for maintenance and repair work on fencing, water developments, and other infrastructure. CSU will reimburse the City or County within thirty (30) days of receipt of a detailed invoice describing the work performed. Estimates for these costs will be provided to CSU for approval prior to the initiation of the work, except in an emergency. In the case of an emergency repair or similar situation, the City or County may proceed with the work and provide invoices for the repair to CSU as soon as reasonably possible.. If Project Funds are depleted and the parties agree that certain maintenance or repair work is necessary and should be performed, CSU may agree to reimburse the cost of such materials provided that the City and County provide the labor for such maintenance or repair work from other sources.

(c) CSU may use the Properties for bison grazing purposes only. This Agreement does not allow for any other private or commercial uses, hunting, shooting, trapping or poisoning of wildlife, or control of prairie dogs.

(d) In consideration of the use of the Properties for grazing, CSU agrees to use best efforts so that any animals that are no longer needed for the Project will be disposed of in priority as follows:

- (1) Provided as "seedstock" to other public conservation herds;
- (2) Sold to private producers, provided all proceeds from such sale are used to support the Project;
- (3) Donated to the City and/or County, subject to the City's or County's acceptance of the donation in its discretion;
- (4) If other options are exhausted, the animals may be sold to slaughter, provided all proceeds from such sale are used to support the Project; or

- (5) If the Project has been terminated or the herd is completely eliminated through slaughter, the proceeds of any sale will be donated to the City and County to reimburse each party for expenses associated with the Project. Once the City and County are fully reimbursed, the remaining funds can be used by CSU to cover Project expenses. Any funds remaining may be donated to the American Bison Society or similar American bison conservation organization.

(e) CSU, in cooperation with the City and County, will conduct studies on how bison grazing impacts prairie ecosystem health. In addition, faculty and students from various colleges will conduct non-invasive research approved by the City and/or County. Participation by other organizations in these studies, such as the Denver Zoo will be managed through CSU. All organizations participating in studies on the Properties must be approved by the Property owner, and approval will be at the Property owner's sole discretion.

6. APHIS Responsibilities. APHIS will provide the "seedstock" bison for the Project and will provide disease monitoring on a regular basis for all Project animals. APHIS will provide testing to ensure that bison remain brucellosis free.

7. Education and Outreach. The parties will collaborate on education and outreach programs related to the Project. All parties will be acknowledged on printed materials, press releases and other materials developed about the Project. Peer-reviewed, scholarly publications will acknowledge all parties but will not give authorship unless individuals provided significant contributions to experimental design, data collection, or data analysis of the published study.

8. Use and Condition of the Properties.

(a) CSU and APHIS acknowledge that the Properties are open to the public. The City and County reserve the right to close all or any portion of the Properties to the public at any time. The City and County also reserve the right to perform management activities on the Properties, and to make alterations, changes and additions to the land and improvements that make up the Properties, at any time.

(b) Neither CSU nor APHIS shall permit or allow the use of the Properties by: (1) the general public, except for members of the public using the Properties in accordance with the City of Fort Collins Code and Natural Areas regulations and policies, and Larimer County Natural Resources Regulations, or (2) any persons other than CSU and APHIS's employees or agents, who are permitted to occupy or use the Properties only to the extent required to carry out the purposes of this Agreement.

(c) Only licensed vehicles are allowed on the Properties and must remain on established roads. Unlicensed vehicles are prohibited, except that ATVs used for animal management activities may be used on or off established roads, but only with the express written permission of the City or County, as applicable, keeping such use to an absolute minimum and only during dry conditions unless such use is necessary due to an emergency situation.



(d) CSU and APHIS acknowledge and agree that the City and County have not made, do not make, and specifically negate and disclaim any representations, warranties, or guarantees of any kind whether expressed or implied, oral or written, past, present, or future, concerning the Properties and; (i) the value, nature, quality, or condition of the Properties, including, without limitation, the water, soil, and geology of the Properties; (ii) the suitability of the Properties for any and all activities and uses which the parties may conduct thereon including the grazing of livestock; (iii) the compliance of or by the Properties or their operation with any laws, rules, ordinances, regulations of any applicable governmental authority or body; (iv) the manner or quality of the construction or materials, if any, incorporated into the improvements located on the Properties; (v) the manner, quality, state of repair or lack of repair of the improvements located on the Properties; or (vi) any other matter with respect to the Properties and the improvements located thereon. Specifically, the City and County have not made, do not make and specifically disclaim any representations regarding compliance with any environmental protection, pollution, or land use laws, rules, regulations, orders, or requirements, including solid waste, as defined by the U.S. Environmental Protection Agency regulated at 40 C.F.R., Part 261, or the disposal or existence, in or on the Properties, of any hazardous substance, as defined by the Comprehensive Environmental Response Compensation and Liability Act of 1980, as amended, and regulations promulgated thereunder.

#### 9. Special Conditions.

(a) Winter/Off Season. CSU, the City and the County acknowledge that access to some or all of the Properties may be difficult in the winter months. CSU, the City and the County agree to use best efforts to monitor bison health, supplemental feed and water needs on the Properties on a regular basis using the resources of CSU, the City and County as available for each Property.

(i) Soapstone Prairie Natural Area. Soapstone has a resident ranch manager that is employed by the grazing tenant on the Property. The City will request that the ranch manager monitor the above needs during his/her regular working hours, even during the period of time that the area is closed to the public. However, the parties acknowledge that the grazing tenant and its ranch manager are not employees or agents of the City and are under no obligation to provide these services. CSU will provide additional support to monitor the animals if the ranch manager is not available and advance notice is provided to CSU by the City.

(ii) Red Mountain Open Space and Reservoir Ridge Natural Areas. CSU, the City and County will agree upon a winter monitoring plan for these two properties prior to the introduction of bison into these areas.

(b) Calving Season. The bison calving season is from April 1<sup>st</sup> – July 31<sup>st</sup> of each year. During this period, visitor access may need to be restricted for public safety, or, at a minimum, additional signage and information may need to be provided to visitors of the Properties. The Parties agree to collaborate to provide this restricted access when warranted.

10. Communications. The Parties agree to collaborate in advance on any press release, media statement, adjacent landowner communication or promotion related to the use of the Property for the Project. Also, the Parties acknowledge that, in the event of an emergency concerning the Property and the Project, they will use their best efforts to coordinate with each other on any public communications; however, coordinating such communications in advance may not be practicable. In addition, the Parties agree to collaborate on their fund raising and communications for the Project.

11. Liability and Insurance.

(a) To the extent permitted by applicable law, each party will be responsible for its own negligent acts or omissions and that of its officers, employees, agents and contractors. Neither the City nor the County will be liable to CSU, APHIS or each other for any livestock injuries or deaths, regardless of cause, incurred in connection with grazing on the Properties under this Agreement, unless such injuries or deaths result from a negligent act or omission of the City or County. Any liability of the City, County, CSU, or their officers and employees is subject to all the defenses, immunities, and limitations of the Colorado Governmental Immunity Act (Section 24-10-101, et seq.) and to any other defenses, immunities, and limitations to liability available under the law.

(b) During the term of this Agreement, CSU, at its sole cost and expense, must procure, pay for, and keep in full force and effect a comprehensive policy of general liability insurance and insuring CSU in an amount not less than One Million Dollars (\$1,000,000.00), which may also be provided through self-insurance, covering bodily injury, including death to persons, personal injury, and property damage liability arising out of a single occurrence. Such coverage must include, without limitation, the insured's liability for property damage, bodily injuries, and death of persons in connection with the keeping of CSU's bison on the Properties (including acts or omissions of CSU or of its officers, employees, or agents), and protection against liability for non-owned and hired automobiles. Such coverage must also include automobile liability insurance. All such policies of insurance must name CSU as an insured and name the City and County as additional insureds. CSU shall provide the City and County with notice of cancellation within 30 days. CSU will provide certificates of insurance to the City and the County as evidence of insurance. Notwithstanding the notice period in paragraph 1, if CSU's insurance is cancelled the City and/or County may immediately terminate this Agreement and require removal of the animals from the Properties.

(c) All Project work conducted on the Properties, including movement of animals from outside Colorado onto the Properties, must comply with all applicable laws, regulations and other legal requirements.

12. Default. If any party defaults in its obligations under the terms of this Agreement, a non-defaulting party may give the defaulting party written notice specifying the nature of the default. If the defaulting party has not cured the default within thirty (30) days, or, for a default reasonable requiring more than 30 days to effect a cure, has not commenced a cure within 30 days and pursued it with diligence, then the non-defaulting party may terminate this Agreement and/or pursue all available remedies at law or in equity.



13. Termination. Upon termination of this Agreement:

(a) All bison must be removed from the Properties unless CSU agrees to donate, and the City or County agrees to accept, some or all of the animals.

(b) Upon termination of the Project, CSU will remove surface fixtures, equipment and other improvements installed on the Properties for the Project, except for the fencing, to the extent requested by the City and County. CSU must consult with the Property owner in advance of any such removal, and the City or County may in its sole discretion require CSU to leave some or all improvements in place, provided that CSU shall be allowed to remove all portable handling facilities from the Properties. If CSU removes improvements from the Properties, CSU shall restore the Properties to a condition comparable to their condition prior to the removal activities.

14. Notices. Any notice or other communication given by any party to another relating to this Agreement must be hand-delivered or sent by registered or certified mail, return receipt requested, or by overnight commercial courier, addressed to such other party at its respective addresses set forth below; and such notice or other communication will be deemed given when so hand-delivered or three (3) business days after so mailed, or the next business day after being deposited with an overnight commercial courier:

If to the City:

Natural Areas Department  
City of Fort Collins  
Attn: Natural Areas Director  
P.O. Box 580  
Fort Collins, CO 80522

With a copy to:

City Attorney's Office  
City of Fort Collins  
P.O. Box 580  
Fort Collins, CO 80522

If to the County:

Larimer County Natural Resources Department  
1800 S. County Road 31  
Loveland, CO 80537

If to CSU:

Animal Reproduction and Biotechnology Lab  
Attn: Department Head  
Colorado State University  
Fort Collins, CO 80523

With a copy to:

Office of the General Counsel  
Colorado State University System  
01 Administration Building  
0006 Campus Delivery  
Fort Collins, CO 80523

If to APHIS:

Animal and Plant Health Inspection Service  
Veterinary Services, Office of the Deputy Administrator  
Washington, DC 20250

15. Obligations Subject to Appropriation. The obligations of the City and of the County to commit or expend funds after calendar year 2014 are subject to and conditioned upon the annual appropriation of funds sufficient and intended to carry out said obligations by the Fort Collins City Council and the Larimer County Board of County Commissioners, respectively, in the City and County's sole discretion. If the City Council or Board of County Commissioners do not appropriate funds necessary to carry out any such obligations, the City or County will notify the other parties promptly of such non-appropriation. If such non-appropriation results in a material impairment of CSU or APHIS's rights hereunder, such party may terminate the lease, with no further recourse against the City or County, by providing thirty (30) days written notice to the City and or County. If neither party exercises this termination right within sixty (60) days of receiving the City's or County's notice of said non-appropriation, then each such party waives its right to terminate this Agreement pursuant to this section.

16. General Provisions.

(a) Words of the masculine gender include the feminine and neuter gender; and when the sentence so indicates, words of the neuter gender refer to any gender. Words in the singular include the plural and vice versa.

(b) This Agreement is to be construed according to its fair meaning and as if prepared by all parties hereto and is deemed to be and contain the entire understanding and agreement between the parties hereto. There shall be deemed to be no other terms, conditions, promises, understandings, statements, or representations, expressed or implied, concerning this Agreement unless set forth in writing and signed by the parties hereto.



(c) This Agreement cannot be modified or assigned except in writing signed by all parties.

(d) Subject to the provisions hereof, the benefits of this Agreement and the burdens hereunder inure to and are binding upon the parties hereto and their respective heirs, administrators, successors, agents and permitted assigns.

(e) This Agreement will be governed by and its terms construed under the laws of the State of Colorado. Any judicial proceedings commenced by a party to enforce any of the obligations, covenants, and agreements contained herein, must be commenced in the Larimer County District or County Courts or the Federal Courts in Denver, Colorado.

(f) Nothing contained herein is deemed or should be construed by the parties nor by any third party as creating the relationship of principle and agent, a partnership or a joint venture between the parties, or an employment relationship between the parties.

(g) This Agreement is made for the sole and exclusive benefit of the City, County, CSU and APHIS, their successors and assigns, and it is not made for the benefit of any third party.

(h) The City and County reserve the right to grant to any third party such easements and rights-of-way as they each may desire over, across, and under portions of the Properties and to lease all or any portion of the Properties to any other third party so long as such easements, rights-of-way, and leases do not unreasonably interfere with the rights of CSU or APHIS as provided in this Agreement.

(i) If any term or condition of this Agreement is held to be invalid by final judgment of any court of competent jurisdiction, the invalidity of such a term or condition, will not in any way affect any of the other terms or conditions of this Agreement, provided that the invalidity of any such term or condition does not materially prejudice any party in their respective rights and obligations under the valid terms and conditions of this Agreement.

(j) To the extent necessary to carry out all of the terms and provisions hereof, the said terms, obligations, and rights set forth herein survive and will not be affected by the expiration or termination of this Agreement.

(k) No party will be deemed in violation of this Agreement if prevented from performing any of its respective obligations hereunder by reason of strikes, boycotts, labor disputes, embargoes, shortage of energy or materials, acts of God, acts of public enemies, acts of superior governmental authorities, weather conditions, rights, rebellions, sabotage, or any other circumstances for which it is not responsible or that are not within its control.

**THE CITY OF FORT COLLINS, COLORADO**  
**a Municipal Corporation**

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Darin A. Atteberry, City Manager

ATTEST:

\_\_\_\_\_  
City Clerk

APPROVED AS TO FORM:

\_\_\_\_\_  
Assistant City Attorney

**LARIMER COUNTY, COLORADO**

By: -----  
Linda Hoffmann, County Manager

APPROVED AS TO FORM:

\_\_\_\_\_  
County Attorney

**THE BOARD OF GOVERNORS OF THE  
COLORADO STATE UNIVERSITY SYSTEM,  
ACTING BY AND THROUGH COLORADO  
STATE UNIVERSITY**

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Amy L. Parsons  
Vice President for University Operations

Approved:

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Dr. Thomas Hansen, Director  
Animal Reproduction and Biotechnology  
Laboratory

Legal Review:

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Jason L. Johnson  
Deputy General Counsel  
Colorado State University System



Animal and Plant  
Health Inspection  
Service

Veterinary Services

1400 Independence  
Ave, SW

Washington, DC  
20250

Date: 2/6/15

*Jack & Shen for John R. Clifford*

John R. Clifford  
Chief Veterinary Officer  
USDA

**Amendment Form  
Animal Care and Use Protocol  
Bison Quarantine Facility Institutional Animal Care and Use Committee**

Study Title:	<b>Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of <i>Brucella abortus</i> in bison</b>
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 GonaCon<sup>TM</sup> 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\_\_\_\_\_



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

## REGULATORY CONSIDERATIONS

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <p>_____</p> <p>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 <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™ 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 \_\_\_\_\_ Date\_\_\_\_\_

Concur

IACUC Chair \_\_\_\_\_ Date\_\_\_\_\_

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

Final ACUC protocol  
5/23/11

**REGULATORY CONSIDERATIONS**

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. ____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011____ Permit(s) description _____ Number _____ Date _____

**DESCRIPTION OF ACTIVITIES**

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

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

Start Date: June 1, 2011

End Date: October 1, 2017

**STUDY PROTOCOL****1. Key Personnel**

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



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

## 2. Testing Facilities

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



serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,





IA.

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% ~~abortion~~ <sup>shedding</sup>). Two replicates of the two pastures will be conducted.

## 11. Animal Care and Use Information

- 1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)  
Breed, strain and substrain (if applicable): NA  
Total Number and Sex: 96 females, 8 males  
Body weight range: 400-1000 kg  
Age: 2 year to adult
- 2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.
- 3) Rationale for appropriateness of the species to be used: Bison are the target species.
- 4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.
- 6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.
- 7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.
- 8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.



9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Carfentanil-0.005-0.01 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Butorphenol- 0.03-0.06 mg/kg, IM dart  
Medetomidine- 0.01-0.02 mg/kg  
Azaperone- 0.02 mg/kg

Reversal for narcotics:

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

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM  
Naltrexone 0.05-0.125mg/kg IM  
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

### 13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

## 12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

## 13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

Attn: Jack K Ryan

Page 9 of 9

Study Protocol

GonaCon-in-bison

PART ONE: SIGNATURE PAGE

Study Director: [Signature]

Date: 5/16/11

Concur: IACUC Chair [Signature] Date 5/16/11

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

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/APHISNS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHISNS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th 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/APHISVS 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 *Bruce/la abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent





on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

## 6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyon JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Bruce/la 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 *Bruce/la 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/APHISNS 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 % mis 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 *Bruce/la* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% shedding). Two replicates of the two pastures will be conducted.

## 11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.



9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart  
Medetomidine- 0.01-0.02 mg/kg  
Azaperone- 0.02 mg/kg

Reversal for narcotics:

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

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM  
· Naltrexone 0.05-0.125mg/kg IM  
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian: ·

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

rgi No

OYes 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 *Bruce/la 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. *Bruce/la 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 *Bruce/la abortus*. J Wildl Dis. 34:582-9.

Attest: Jacki K. Brown  
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PART ONE: SIGNATURE PAGE

study oirectoc

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Date: *;;Jc;;b*  
7 7

Concur: \_\_\_\_\_  
ACUC Chair \_\_\_\_\_, t Jk.c



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

## REGULATORY CONSIDERATIONS

Permits					
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <table style="width: 100%; border: none;"> <tr> <td style="border: none; width: 50%;">Permit(s) description</td> <td style="border: none; width: 30%;">Number</td> <td style="border: none; width: 20%;">Date</td> </tr> </table>	Permit(s) description	Number	Date
Permit(s) description	Number	Date			

## DESCRIPTION OF ACTIVITIES

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

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

Start Date: June 1, 2011

End Date: October 1, 2017

## STUDY PROTOCOL

### 1. Key Personnel

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

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

## 2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19 <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 anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

## 8. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by

serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% shedding). Two replicates of the two pastures will be conducted.

## 11. Animal Care and Use Information

1) Animal Information: Species, subspecies (if applicable): Bison (*Bison bison*)

Breed, strain and substrain (if applicable): NA

Total Number and Sex: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

2) Rationale for involving animals: This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

3) Rationale for appropriateness of the species to be used: Bison are the target species.

4) Source: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

5) Method of identification of animals: Animals will be ear tagged and microchipped for identification.

6) Trapping/Collecting: Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

7) Transport: Animals will be loaded on to stock trailers and transported to the Corwin Springs facility.

8) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana.

9) Handling/restraint: Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart

Carfentanil-0.005-0.01 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart

Butorphenol- 0.03-0.06 mg/kg, IM dart  
Medetomidine- 0.01-0.02 mg/kg  
Azaperone- 0.02 mg/kg

Reversal for narcotics:

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

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM  
Naltrexone 0.05-0.125mg/kg IM  
Tolazoline 1 mg/kg IM

10) Disposition of animals: It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

11) Animal pain or distress

Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.



Name of Attending Veterinarian: Patrick Ryan Clarke

Date of Consultation: 13 May 2011

12) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

- a) Alternative procedures:
- b) Sedatives, analgesics, or anesthetics or Column E Explanation:
- c) Surgery:

### 13) Euthanasia

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

### 12. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

### 13. References

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

Rankin, J. E., 1965. *Brucella abortus* in bulls: a study of twelve naturally infected cases. Vet Rec. 77:132-5.

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.

PART ONE: SIGNATURE PAGE

Study Director: [Signature] Date: 5/16/11

Concur: IACUC Chair [Signature] Date 5/16/11

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
<b>Study Director</b>		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
<b>Other Investigators, Collaborators, Cooperators, and Consultants</b>		
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.

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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.

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Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,



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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: 96 females, 8 males

Body weight range: 400-1000 kg

Age: 2 year to adult

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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:
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**SIGNATURE PAGE**

Study Director

Jade C. Pyper

Date 5/16/2011

Concur

IACUC Chair

\_\_\_\_\_

Date \_\_\_\_\_

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

## REGULATORY CONSIDERATIONS

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## DESCRIPTION OF ACTIVITIES

- Nature of the Collaboration:
- ☐ *Advisory Committee participation*
  - ☒ *Manuscript/review article collaboration*
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	Rick Wallen, Jenny Powers	National Park Service	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Start Date: June 1, 2011

End Date: October 1, 2017

## STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
<b>Study Director</b>		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
<b>Other Investigators, Collaborators, Cooperators, and Consultants</b>		
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™ 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

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**SIGNATURE PAGE**

Study Director

Jade C. Ryan

Date

5/16/2011

Concur

IACUC Chair

\_\_\_\_\_

Date

\_\_\_\_\_

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

## REGULATORY CONSIDERATIONS

Permits					
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <p>_____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____</p> <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
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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 <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

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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.

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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:
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**PART ONE: SIGNATURE PAGE**

Study Director:



Date:

5/16/11

Concur:

IACUC Chair

Date

## 1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services  
National Wildlife Research Center

### PROTOCOL COVER PAGE

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

<b>1</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, <b>and</b> there is generally no commitment of NWRC resources other than personnel time, <b>and</b> activities are not regulated research activities.</p> <p><u>Complete &amp; 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"> <li>• Writing or collaborating on review papers and synthesis reports</li> <li>• Student committee participation</li> <li>• Analyzing or writing up data collected under operational or other contexts</li> </ul>
<b>2</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, <b>but</b> the activity involves regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; 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"> <li>• Training programs requiring the use of animals</li> <li>• Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)</li> <li>• Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)</li> </ul>
<b>3</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>but</b> the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• Collaborating on study design, data analysis, or economic analysis.</li> <li>• Minor participation on a regulated study at the collaborating host institution</li> <li>• A study that does not include animal use, etc.</li> </ul>
<b>4</b> <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>and</b> the study includes regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• A typical NWRC led study</li> <li>• Major NWRC staff participation in regulated activity</li> <li>• Study takes place on NWRC facilities</li> </ul>

\* 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".

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## PART ONE: SIGNATURE PAGE

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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 \_\_\_\_\_

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Note: Additional approvals are located in the attached appendices.

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## PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
<b>Animal Use</b>		
<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 <b>NWRC Animal Use Appendix</b> <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach <b>IACUC protocol &amp; approval</b> <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.
<b>Microbiological/Biohazardous Materials</b>		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach <b>Microbiological/Biohazardous Materials Use Appendix</b> .
<b>Permits</b>		
<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 _____
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>		
<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 <b>NEPA &amp; ESA Appendix</b> .
<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 <b>NEPA &amp; ESA Appendix</b> . 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.
<b>Regulatory Standard and Test Guidelines</b>		
<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: _____
<b>Test, Control and Reference Material/Devices</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the <b>Test, Control and Reference Material/Devices Formulation and Use Appendix</b> . Please indicate if otherwise described in the protocol.
<b>Historical Resources</b>		
<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.
<b>Material Transfer Agreement</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate <b>Material Transfer Agreement</b> . Material Transfer agreements will be developed prior to material transfer
<b>Analytical Chemistry</b>		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?

	If yes, attach <b>Analytical Chemistry Appendix</b> .
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## PART FOUR: FULL NWRC STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
<b>Study Director</b>		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
<b>Other Investigators, Collaborators, Cooperators, and Consultants</b>		
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 <sup>th</sup> and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

### 3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

### 4. Schedule

Proposed Experimental Start Date: April 15, 2012  
 Proposed Experimental Termination Date: October 1, 2017  
 Proposed Study Completion/Archive Date: October 1, 2019

### 5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

## 6. Related Protocols

- |      |                                                                                                                                                                             |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer ( <i>Odocoileus virginianus</i> ): Pivotal target animal safety study                                             |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission                                                                                                 |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland                                                    |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey                                                  |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland              |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer ( <i>Dama dama</i> ) at Point Reyes National Seashore, California            |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk ( <i>Cervus elaphus</i> ) at Rocky Mountain National Park, Colorado                  |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses ( <i>Equus caballus</i> ) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer                                                                                                                                 |

## 7. Assurance of Non-Duplication of Studies

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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

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### 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

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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.

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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 <sup>st</sup> replicate			
2012	Collect bison for 1 <sup>st</sup> and 2 <sup>nd</sup> replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 <sup>nd</sup> replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 <sup>nd</sup> replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

## 11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

## 12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a. Animal handling and sample collection records
  - b. Necropsy records
  - c. Results of serologic, histopathologic, and cultural analysis
  - d. Animal calving observation records
  - e. Pregnancy assessment records
- D. Final Report

## 13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and 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 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.

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## 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

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## 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.

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## 18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer



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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

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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

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## Animal Use Appendix

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**A). Animal Information:**

Species, subspecies (if applicable): Bison (*Bison bison*)  
Breed, strain and substrain (if applicable): NA  
Total Number and Sex: 96 females, 8 males  
Body weight range: 400-1000 kg  
Age: 2 year to adult

**B1) Rationale for involving animals:**

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

**B2) Rationale for numbers to be used:** If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

**B3) Rationale for appropriateness of the species to be used:** Bison are the target species.

**C) Source:** Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

**D) Method of identification of animals:** Animals will be ear tagged and microchipped for identification.

**E) Trapping/Collecting:** Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

**F) Transport:** Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

**G) Handling/restraint:** Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Carfentanil-0.005-0.01 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Butorphenol- 0.03-0.06 mg/kg, IM dart  
Medetomidine- 0.01-0.02 mg/kg  
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

**J) Dietary contaminant exposure NA**

**K) Disposition of animals:** It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

**L) Animal pain or distress**

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: \_\_\_\_ Patrick Ryan Clarke\_\_\_\_

Date of Consultation: \_\_\_\_ 13 May 2011\_\_\_\_

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

**M) Euthanasia**

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

**N. IACUC Approval**

Date of IACUC Approval Letter: \_\_ACUC Protocol approved 5/17/2011\_ See attached\_\_

Bison Quarantine Facility Institutional Animal Care and Use Committee

**O. Staff Qualifications**

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

## NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

**Effects on T&E species and eagles:**

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

**Consultations:**

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyon has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

## Test, Control and Reference Material/Devices Formulation and Use Appendix

### A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
  - a) Concentration and purity: 1000ug/ml purity:na
  - b) Source: National Wildlife Research Center
  - c) Batch number: to be determined

### B. Describe any control or reference materials/devices

No control or reference materials will be used

### C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

### D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

### G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

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

<b>1</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, <b>and</b> there is generally no commitment of NWRC resources other than personnel time, <b>and</b> activities are not regulated research activities.</p> <p><u>Complete &amp; 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"> <li>Writing or collaborating on review papers and synthesis reports</li> <li>Student committee participation</li> <li>Analyzing or writing up data collected under operational or other contexts</li> </ul>
<b>2</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, <b>but</b> the activity involves regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; 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"> <li>Training programs requiring the use of animals</li> <li>Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)</li> <li>Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)</li> </ul>
<b>3</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>but</b> the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>Collaborating on study design, data analysis, or economic analysis.</li> <li>Minor participation on a regulated study at the collaborating host institution</li> <li>A study that does not include animal use, etc.</li> </ul>
<b>4</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>and</b> the study includes regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>A typical NWRC led study</li> <li>Major NWRC staff participation in regulated activity</li> <li>Study takes place on NWRC facilities</li> </ul>

\* 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".



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## PART ONE: SIGNATURE PAGE

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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 \_\_\_\_\_

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Note: Additional approvals are located in the attached appendices.

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## PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
<b>Animal Use</b>		
<input type="checkbox"/>	<input 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 <b>NWRC Animal Use Appendix</b> <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach <b>IACUC protocol &amp; approval</b> <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.
<b>Microbiological/Biohazardous Materials</b>		
<input type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach <b>Microbiological/Biohazardous Materials Use Appendix</b> .
<b>Permits</b>		
<input type="checkbox"/>	<input 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; border-top: 1px solid black; margin-top: 10px;"> <span>Permit(s) description</span> <span>Number</span> <span>Date</span> </div>
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>		
<input type="checkbox"/>	<input 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 <b>NEPA &amp; ESA Appendix</b> .
<input 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 <b>NEPA &amp; ESA Appendix</b> . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input type="checkbox"/>	<input type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input type="checkbox"/>	<input type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
<b>Regulatory Standard and Test Guidelines</b>		
<input 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 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 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: _____
<b>Test, Control and Reference Material/Devices</b>		
<input type="checkbox"/>	<input type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the <b>Test, Control and Reference Material/Devices Formulation and Use Appendix</b> . Please indicate if otherwise described in the protocol.
<b>Historical Resources</b>		
<input 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.
<b>Material Transfer Agreement</b>		
<input 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 <b>Material Transfer Agreement</b> .
<b>Analytical Chemistry</b>		
<input type="checkbox"/>	<input type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach <b>Analytical Chemistry Appendix</b> .

**PART THREE: DESCRIPTION OF ACTIVITIES**

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

Collaboration:	Name	Address or Organization	Role in Project

Start Date:

End Date:

Archive Date:

Anticipated Project Outcome: ☐ Manuscript  
☐ Report  
☐ Other: \_\_\_\_\_

Materials to be archived to close this activity:

Description of Project and NWRC Activities and Participation:

Comments:

Attachments:  
(e.g. Material Transfer Form, IACUC approval, etc.)

## PART FOUR: FULL NWRC STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
Study Director		
Other Investigators, Collaborators, Cooperators, and Consultants		

### 2. Testing Facilities

Name	Address	Role in Study

### 3. Sponsor

Name	Address	Contract No.

### 4. Schedule

Proposed Experimental Start Date:  
Proposed Experimental Termination Date:  
Proposed Study Completion/Archive Date:

### 5. Background and Justification

Give the rationale for the study with an analysis of the problem situation and a clear statement of need and justification. Include a summary of the literature reviewed.

### 6. Related Protocols

List by Protocol Number

### 7. Assurance of Non-Duplication of Studies

Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.

## 8. Objective/Hypotheses

Give concise statements as to the objective of the study and the hypotheses to be tested.

## 9. Methods/Procedures

Give a logical sequence of events leading toward attainment of the objectives including the type and frequency of tests, measurements, and analyses to be made. The level of detail should be at a level which would allow an independent third party or educated lay person to read and conceptually understand it and a scientific researcher to conduct or repeat the study based solely on the protocol. For field studies include a description of the field sites where the study will be conducted. Refer to details in the attached appendices as appropriate. Analytical chemistry procedures may be indicated in the attached appendices, but all other methods and procedures must be provided directly or by reference to the appropriate SOP(s). Information frequently forgotten includes randomization schemes and procedures, bioanalytical assays, and a comprehensive description of all procedures and methods (field and lab), etc.

## 10. Experimental Design and Statistical Analyses

Describe the experimental design including methods for control of bias. Include sample sizes, sketches, and narrative as needed to make the design clear. Give a statement of the proposed statistical method or methods to be used. If a statistician was consulted for assistance in study design, give the date of the consultation and the name and affiliation of the person consulted.

## 11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title

## 12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a.
  - b.
  - c.
  - d.
- D. Final Report
- E. \_\_\_\_\_

### 13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx		
A. Salary and Benefits					
B. Facilities (in addition to existing facility or space costs)					
C. Equipment					
D. Supplies					
E. Animal Care Costs					
F. Operating Costs (travel, misc. services, etc)					
TOTAL	\$0	\$0	\$0		

### 14. Human Health and Safety

Cite the appropriate SOP(s) or explain briefly the safety precautions, equipment, and procedures to be used for potentially hazardous conditions. State whether or not the proposed research has any potential for risk to the health or safety to members of the public, and, if so, explain how such risk(s) will be minimized or avoided.

### 15. Staff Qualifications

*[Standard text revise as needed]* All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study.

### 16. Archiving

*[Standard text revise as needed]* All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

### 17. Protocol Amendments

*[Standard text revise as needed]* Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

### 18. References

List in alphabetical order by author.

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## 19. Appendices

Indicate none or check attached appendices:

- ☐ None
  - ☐ Animal Use Appendix
  - ☐ Analytical Chemistry Appendix
  - ☐ Column E Explanation
  - ☐ Material Transfer Agreement
  - ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
  - ☐ NEPA and ESA Appendix
  - ☐ Test, Control and Reference Material/Device Use Appendix
  - ☐ Other: (include appropriate title) \_\_\_\_\_
  
  - ☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached
-

## Animal Use Appendix

An “Animal” is defined as any vertebrate. “Use” includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

### A. Animal Description

#### 1) Animals:

Species, subspecies (if applicable):

Breed, strain and substrain (if applicable):

Total Number and Sex:

Body weight range:

Age:

**B. Rationale for involving animals, for appropriateness of species, and for numbers** Provide justification why this study requires the use of animals, and for the numbers to be used.

#### 1) Rationale for involving animals:

#### 2) Rationale for appropriateness of the species to be used:

#### 3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):

### C. Source

Describe where the animals will be trapped or obtained, or identify the vendor by name and address.

### D. Method of identification of animals

Cite the appropriate SOP(s) or explain briefly how animals will be marked or identified to prevent misidentification.

### E. Trapping/Collecting

Cite the appropriate SOP(s) or explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.)

### F. Transport

Cite the appropriate SOP or explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, etc.



**G. Handling/restraint**

Cite the appropriate SOP(s) or explain briefly how the animals will be held or restrained (manual vs. chemical) throughout study.

**H. Quarantine**

Cite the appropriate SOP, or describe the procedure for the quarantine of animals.

**I. Housing/maintenance**

Cite the appropriate SOP(s) or explain briefly how housing/maintenance will be done (including information on feeder animals if used).

**J. Dietary contaminant exposure**

Are there any contaminants or diet supplements that are reasonably expected to be present in the dietary materials, drinking water, or bedding material and are known to be capable of interfering with the purpose or conduct of the study? If so, please describe control/testing mechanism.

**K. Disposition of animals**

Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study, or cite the appropriate SOP(s).

**L. Animal pain or distress****1) Consultation with Attending Veterinarian:**

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

**Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.**

Name of Attending Veterinarian: \_\_\_\_\_

Date of Consultation: \_\_\_\_\_

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☐ No

☐ Yes If yes, continue with the following items.

**a) Alternative procedures:**

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

**M. Euthanasia**

Describe the appropriate method of euthanasia to be used (cite the appropriate SOP or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)

**N. IACUC Approval**

Date of IACUC Approval Letter: \_\_\_\_\_

**O. Staff Qualifications**

List the study participants that will be working independently with animals and provide their qualifications/certifications (i.e. name, title, and a brief description of training/experience).

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## Analytical Chemistry Appendix

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If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

- A. Number of samples to be analyzed (by type):**
- B. Storage conditions (temperature, container type, light/dark, duration):**
- C. Method title and number:**
- D. ACP Leader approval: \_\_\_\_\_ Date: \_\_\_\_\_**  
(attach email or letter of concurrence from Analytical Services Project Team Leader)

If chemical analysis will be made by a laboratory outside of NWRC, include A-C above and attach the method to be used.

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### Column E Explanation

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1. Registration Number: 84-F-0001
2. Number of animals used in this study during this reporting period:
3. Species (common name) of animals used in study during this reporting period:
4. Explain procedure producing pain and/or distress:
5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):
6. What, if any, federal regulations require this procedure?

Agency:                      CFR:

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## Material Transfer Agreement

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**STANDARD AGREEMENT**  
**U. S. Department of Agriculture**  
**Animal and Plant Health Inspection Service**  
**National Wildlife Research Center**

**PARTIES:**

APHIS:           USDA, APHIS  
                    National Wildlife Research Center  
                    Scientist Address  
                    City, State Zip  
                    Tel: Telephone # of Scientist  
                    FAX: FAX # of Scientist  
                    E-Mail: E-mail address of Scientist

Recipient:       Company Name  
                    Company Address  
                    City, State Zip of Company  
                    Tel: Telephone # of Recipient  
                    FAX: FAX # of Recipient  
                    E-mail: E-mail address of Recipient

**PURPOSE:**

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

*[Table may be adjusted as needed]*

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material, shall provide APHIS with any manuscript that describes the work with the Material and shall acknowledge APHIS' contribution to the work reported when appropriate.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's

use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.

7. Upon completion of the activities performed using the Material, the Material shall be [redacted] *[for example, returned to ..., destroyed by..., disposed of as instructed by APHIS].*

8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated].* THIS MATERIAL MAY BE INFECTED WITH PATHOGENS INCLUDING, BUT NOT LIMITED TO, *[NAME OF PATHOGEN]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.

9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed of.

QA#:	Permit Information (Type and Number):
------	------------------------------------------

**ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:**

_____ Typed name/Title	_____ Signature (NWRC Scientist)	_____ Date
_____ Typed Name/Title	_____ Signature (NWRC Project Leader)	_____ Date

**APHIS APPROVING OFFICIAL:**

_____ Typed Name/Title	_____ Signature (NWRC Assistant Director)	_____ Date
---------------------------	----------------------------------------------	---------------

**ACCEPTED FOR RECIPIENT:**

_____ Typed Name/Title	_____ Signature	_____ Date
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Original: Quality Assurance Unit

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## Material Transfer Agreement

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### ANIMAL / ANIMAL TISSUE TRANSFER AGREEMENT

U. S. Department of Agriculture  
Animal and Plant Health Inspection Service  
National Wildlife Research Center

#### **PARTIES:**

APHIS: USDA, APHIS  
National Wildlife Research Center  
Scientist Address  
City, State Zip  
Tel: Telephone # of Scientist  
FAX: FAX # of Scientist  
E-Mail: E-mail address of Scientist

**Recipient:** Company Name  
Company Address  
City, State Zip of Company  
Tel: Telephone # of Recipient  
FAX: FAX # of Recipient  
E-mail: E-mail address of Recipient

#### **PURPOSE:**

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

*[Table may be adjusted as needed]*

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material, shall provide APHIS with any manuscript that describes the work with the Material and shall acknowledge APHIS' contribution to the work reported when appropriate.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.

6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.

7. Upon completion of the activities performed using the Material, the Material shall be [redacted] *[for example, returned to ..., destroyed by....., disposed of as instructed by APHIS].*

8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated].* THIS MATERIAL MAY BE INFECTED WITH PATHOGENS INCLUDING, BUT NOT LIMITED TO, *[NAME OF PATHOGEN]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.

9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed of.

QA#:	Permit Information (Type and Number):
------	------------------------------------------

**ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:**

_____ Typed name/Title	_____ Signature (NWRC Scientist)	_____ Date
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_____ Typed Name/Title	_____ Signature (NWRC Project Leader)	_____ Date
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**APHIS APPROVING OFFICIAL:**

_____ Typed Name/Title	_____ Signature (NWRC Assistant Director)	_____ Date
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**ACCEPTED FOR RECIPIENT:**

_____ Typed Name/Title	_____ Signature	_____ Date
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Original: Quality Assurance Unit



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## Microbiological/Biohazardous Materials Use Appendix

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NWRC proposed research or testing activities which involve the use of microbiological organisms or biohazardous agents at or above a Biosafety Level 2 or Risk Level 2, or use recombinant DNA *in vivo*, require this appendix to be completed and submitted to the NWRC IBC for review and approval.

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at [www.cdc.gov/od/ohs/biosfty/biosfty.htm](http://www.cdc.gov/od/ohs/biosfty/biosfty.htm) for the definitions and lists of BioSafety Level 2 organisms and above.

Reference the American Biological Safety Association's (ABSA) "Risk Group Classification for Infectious Agents" at <http://www.absa.org/resriskgroup.html> for the definitions and lists of Risk Level 2 agents and above.

Reference the National Institute of Health's (NIH) Guidelines for Recombinant DNA and Gene Transfer at [www4.od.nih.gov/oba/rac/documents1.htm](http://www4.od.nih.gov/oba/rac/documents1.htm) for specific practices for constructing and handling recombinant DNA and organisms/viruses containing recombinant DNA molecules. Definition of recombinant DNA; 1) Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or 2) Molecules that result from the replication of those in 1 above.

**A. Identify the organism(s)/agent to be used (e.g., species, strain, type, etc.):**

**B. Is this a Select Agent (see [www.selectagents.gov/agentToxinList.htm](http://www.selectagents.gov/agentToxinList.htm))?**

**C. Does the organism contain recombinant DNA, or will recombinant DNA be constructed *in vivo* as a biologically active polynucleotide or polypeptide product? If yes, then address each of the following (if no, then N/A):**

1. The source(s) of the DNA.
2. The nature of the inserted DNA sequences.
3. The host(s) and vector(s) to be used.
4. Will an attempt be made to obtain expression of a foreign gene? If so, indicate the protein that will be produced.
5. The containment conditions that will be implemented.

**D. Source of the organism(s)/agent (e.g., location or name and address of lab/vendor):**

**E. Procedures for shipping and transportation (e.g., from facility to facility, and from room to room):**

**F. Location(s) where the materials are to be used and stored (include all buildings and room number and laboratories):**

**G. Permit information:**

**H. Inventory and tracking procedures (e.g., chain of custody procedures):**

**I. Quality control measures (e.g., procedures to prevent contamination of stocks):**

**Agent Hazards:**

**J. What particular hazards to humans, animals, and the environment are associated with these organisms/agents?** (e.g., infective dose, severity of disease, mode of transmission, susceptibility to humans, stability in the environment, etc.)

**Laboratory Procedure Hazards:**

**K. Estimated volume, amount or concentration of agents or solutions:**

**L. Identify known or potential sources of contamination or exposure** (e.g., infected live animals, tissues, fluids, byproducts, waste, sharps, etc.)

**M. Identify any procedures and equipment which could produce aerosols** (e.g., pipetting, blenders, centrifuges, sonication and vortexing), and describe how the creation of aerosols and/or exposures to those aerosols will be minimized.

**Biosafety, Security and Additional Precautions:**

**N. Biosafety Level / Risk Level (from the CDC or ABSA reference above):**

**O. Biosecurity Plan** (the Biosecurity Plan is a description of a number of different aspects which together define the mechanisms by which biohazardous agents will be safely and securely used)

**1. Physical Security:** Describe procedures to prevent unauthorized access or use of the organisms/materials.

**2. Biosecurity:** Describe the procedures, processes, facility controls and equipment that will be used to ensure biosecurity. Include but not limited to: Description of containment; Bio-inclusion (procedures to keep biological agents in containment); Bio-exclusion (procedures to keep unwanted biological agents out of containment); Decontamination (including work surfaces, materials, cages, equipment, rooms, etc.); and Disposal procedures, including carcass disposal.

**P. Specialized Risk Control Measures:**

Describe specialized risk control measures to be used to protect personnel and prevent exposures. Describe items that are specific or unique for this study (e.g., personal protective equipment, immunizations or medical surveillance, training, or other specialized precautions, equipment, or practices).

**T. Provide an assurance statement that all practices and procedures are in accordance with the appropriate guidelines for that biosafety/risk level of organism/materials:**

**U. NWRC Institutional Biosafety Committee (IBC):**

Date of IBC approval letter:\_\_\_\_\_

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## NEPA and ESA Appendix

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A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
  - ☐ B) not cause contaminants to enter water bodies
  - ☐ C) not adversely affect any federally protected species or critical habitat
  - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☐ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

**Effects on T&E species and eagles:**

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☐ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

**Consultations:**

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

## Test, Control and Reference Material/Devices Formulation and Use Appendix

### A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code
  - a) Concentration and purity:
  - b) Source:
  - c) Batch number:

For non-standard materials, describe the material/device in detail and provide the name and location of the formulation laboratory or facility that will prepare the material.

### B. Describe any control or reference materials/devices

As above, for each material provide the chemical, bait or device

- 1) name or code
  - a) Concentration and purity:
  - b) Source:
  - c) Batch number:

### C. Carriers, mixtures and material preparation

Give a full description of any carriers for the test/reference substance, mixing procedures, bait formulation procedures and a full description of possible contaminants and acceptable ranges for them. Include solvents, emulsifiers, dietary/bait materials and/or other materials used to dissolve or suspend the test or control substances.

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: \_\_\_\_\_ Date: \_\_\_\_\_

### D. Route of administration

Describe the route of administration of the test substance and give a reason for its selection.

### E. Dosage

Define the dose levels of the test or control substances in appropriate units of measurement, and the frequency of administration.

### F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): \_\_\_\_\_

**G. Material verification**

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

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

ACP Consultation: \_\_\_\_\_ Date: \_\_\_\_\_

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 transmission of <i>Brucella abortus</i> in bison
NWRC Study Director:	Jack Rhyan, Lowell Miller
Approved NWRC Project:	

**PROTOCOL CLASSIFICATION**

<b>1</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, <b>and</b> there is generally no commitment of NWRC resources other than personnel time, <b>and</b> activities are not regulated research activities.</p> <p><u>Complete &amp; 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"> <li>• Writing or collaborating on review papers and synthesis reports</li> <li>• Student committee participation</li> <li>• Analyzing or writing up data collected under operational or other contexts</li> </ul>
<b>2</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, <b>but</b> the activity involves regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; 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"> <li>• Training programs requiring the use of animals</li> <li>• Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)</li> <li>• Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)</li> </ul>
<b>3</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>but</b> the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• Collaborating on study design, data analysis, or economic analysis.</li> <li>• Minor participation on a regulated study at the collaborating host institution</li> <li>• A study that does not include animal use, etc.</li> </ul>
<b>4</b> <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>and</b> the study includes regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• A typical NWRC led study</li> <li>• Major NWRC staff participation in regulated activity</li> <li>• Study takes place on NWRC facilities</li> </ul>

\* 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".

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**PART ONE: SIGNATURE PAGE**

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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 \_\_\_\_\_

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Note: Additional approvals are located in the attached appendices.

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## PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item									
<b>Animal Use</b>											
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.</p> <p><input checked="" type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix</p> <p><input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol &amp; approval</p> <p><input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.</p>									
<b>Permits</b>											
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)?</p> <p>If yes, list the legal authority, animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, State Wildlife agency permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, other registrations, etc. under which the study will be conducted. Include all required permit numbers and approval dates.</p> <table border="0"> <tr> <td>_____</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>_____</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>Permit(s) description</td> <td>Number</td> <td>Date</td> </tr> </table>	_____	_____	_____	_____	_____	_____	Permit(s) description	Number	Date
_____	_____	_____									
_____	_____	_____									
Permit(s) description	Number	Date									
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>											
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA &amp; ESA Appendix.</p>									
	<input checked="" type="checkbox"/>	<p>Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA &amp; ESA Appendix. Contact QA/NEPA staff for ESA or eagle incidental take requirements.</p>									
	<input checked="" type="checkbox"/>	<p>Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.</p>									
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<p>Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.</p>									
<b>Regulatory Standard and Test Guidelines</b>											
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Will this study be conducted under any regulatory standard? If yes please check:</p> <p><input checked="" type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</p> <p><input type="checkbox"/> Other:</p>									
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:</p>									
<b>Test, Control and Reference Material/Devices</b>											
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix. Please indicate if otherwise described in the protocol.</p>									
<b>Historical Resources</b>											
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<p>Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices on historic buildings or creating noise impact on historic places)? If yes, provide information. Consultation with the State Historic Preservation Office will be required.</p>									
<b>Material Transfer Agreement</b>											
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<p>Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement.</p>									
<b>Analytical Chemistry</b>											
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<p>Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?</p> <p>If yes, attach Analytical Chemistry Appendix.</p>									

Commented [pn1]:

Commented [pn2]:

Commented [pn3]:

Commented [pn4]:

### PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	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: ☒ Manuscript  
☐ Report  
☐ Other: \_\_\_\_\_

Anticipated Project Outcome: 1. Determine the effectiveness of GonaCon vaccine in reducing transmission of *B. abortus* in bison herds.  
 2. Determine the effect of prolonged anestrus due to GonaCon vaccine on *B. abortus* survival in infected bison.  
 3. Determine the risk and extent of exposure of bison to *B. abortus* at parturition sites.  
 4. Determine the nature of infection (transient or ongoing) in calves due to suckling of seropositive cows.  
 5. Examine the risk of *B. abortus* venereal transmission to seronegative bulls.

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.)

## PART FOUR: FULL NWRC STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
<b>Study Director</b>		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
<b>Other Investigators, Collaborators, Cooperators, and Consultants</b>		
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	Corwin Springs, MT	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	Corwin Springs, MT	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	Bozeman, MT	Fetus sample collection and incineration
National Veterinary Services Laboratory	Ames, IA	Serologic testing and culture of collected samples
National Wildlife Research Center	Fort Collins, CO	Serologic testing

Commented [pn5]: Where will the vaccine be manufactured?

### 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

List by Protocol Number

Commented [pn6]:

## 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

Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.

## 8. Objective/Hypotheses

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 anestrous will have no effect on *B. abortus* colonization in naturally-infected female bison.

**Give concise statements as to the objective of the study and the hypotheses to be tested.**

## 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 (Rhyar

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

## 12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a.
  - b.
  - c.
  - d.
- D. Final Report
- E. \_\_\_\_\_



### 13. Cost Estimate for Each Fiscal Year

	FY-xx	FY-xx	FY-xx		
A. Salary and Benefits					
B. Facilities (in addition to existing facility or space costs)					
C. Equipment					
D. Supplies					
E. Animal Care Costs					
F. Operating Costs (travel, misc. services, etc)					
TOTAL	\$0	\$0	\$0		

### 14. Human Health and Safety

*[Standard text revise as needed]* All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study.

### 15. Staff Qualifications

*[Standard text revise as needed]* All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

### 16. Archiving

*[Standard text revise as needed]* Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistance Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

### 17. Protocol Amendments

*[Standard text revise as needed]* Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistance Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

### 18. References

List in alphabetical order by author.

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## 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: This study requires the use of animals as the project's objectives are to determine the effect of immunocontraception on natural transmission of *Brucella abortus* in a live animal model.

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 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%. The treatment will be deemed successful if the number of seroconversions in the nonvaccinated group exceeds the number of seroconversions in the treated group by 50% or more. Since sample sizes in these types of experiments are invariably small, we consider an alpha level of 0.1 to be acceptable in order to diminish the risk of Type II error in such a costly trial. 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 and alpha constraints.

#### C. Source

Describe where the animals will be trapped or obtained, or identify the vendor by name and address.

#### D. Method of identification of animals

Animals will be ear tagged and microchipped for identification

**E. Trapping/Collecting**

Cite the appropriate SOP(s) or explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.)

**F. Transport**

Cite the appropriate SOP or explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, etc.

**G. Handling/restraint**

Cite the appropriate SOP(s) or explain briefly how the animals will be held or restrained (manual vs. chemical) throughout study.

**H. Quarantine**

Cite the appropriate SOP, or describe the procedure for the quarantine of animals.

**I. Housing/maintenance**

Cite the appropriate SOP(s) or explain briefly how housing/maintenance will be done (including information on feeder animals if used).

**J. Dietary contaminant exposure**

NA

**K. Disposition of animals**

Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study, or cite the appropriate SOP(s).

**L. Animal pain or distress**

1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

**Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.**

Name of Attending Veterinarian: \_\_\_\_\_

Date of Consultation: \_\_\_\_\_

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☐ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

#### **M. Euthanasia**

Describe the appropriate method of euthanasia to be used (cite the appropriate SOP or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)

#### **N. IACUC Approval**

Date of IACUC Approval Letter: \_\_\_\_\_

#### **O. Staff Qualifications**

List the study participants that will be working independently with animals and provide their qualifications/certifications (i.e. name, title, and a brief description of training/experience).

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### Analytical Chemistry Appendix

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If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

- A. Number of samples to be analyzed (by type):**
- B. Storage conditions (temperature, container type, light/dark, duration):**
- C. Method title and number:**
- D. ACP Leader approval:** \_\_\_\_\_ **Date:** \_\_\_\_\_  
(attach email or letter of concurrence from Analytical Services Project Team Leader)

If chemical analysis will be made by a laboratory outside of NWRC, include A-C above and attach the method to be used.

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**Column E Explanation**

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1. Registration Number: 84-F-0001
2. Number of animals used in this study during this reporting period:
3. Species (common name) of animals used in study during this reporting period:
4. Explain procedure producing pain and/or distress:
5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):
6. What, if any, federal regulations require this procedure?

Agency:                      CFR:

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## Material Transfer Agreement

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**STANDARD AGREEMENT  
U. S. Department of Agriculture  
Animal and Plant Health Inspection Service  
National Wildlife Research Center**

**PARTIES:**

APHIS:       USDA, APHIS  
              National Wildlife Research Center  
              Scientist Address  
              City, State Zip  
              Tel: Telephone # of Scientist  
              FAX: FAX # of Scientist  
              E-Mail: E-mail address of Scientist

Recipient:    Company Name  
              Company Address  
              City, State Zip of Company  
              Tel: Telephone # of Recipient  
              FAX: FAX # of Recipient  
              E-mail: E-mail address of Recipient

**PURPOSE:**

To provide Recipient with [REDACTED] and associated know how, hereinafter collectively referred to as the Material.

The Material is released to Recipient under the following conditions:

1. The Material and associated know-how shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall remain the property of APHIS and shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material and shall provide APHIS with any manuscript that describes the work with the Material prior to submission for publication and acknowledge APHIS' contribution to the work reported.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Center for Disease Control, and /or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.
7. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.



8. Upon completion of the activities performed using the Material, the Material shall be returned, destroyed or otherwise disposed of as instructed by APHIS.
9. Recipient shall meet with U.S. Department of Agriculture representatives to determine inventorship if an invention should arise from work with the Material.
10. Recipient shall not disclose Material marked "Confidential" or "Proprietary" to any third party without written permission from APHIS.
11. Material shall be excluded from the confidentiality requirements of this Agreement if: (1) Recipient had possession of the Material prior to disclosure; (2) the Material is generally available to the public at the time of disclosure; (3) the information becomes generally available to the public through no fault of Recipient after disclosure; or (4) after disclosure, Recipient receives the Material from a third party having the right to the Material and who does not impose a confidentiality obligation upon Recipient.
12. If the parties hereto decide, at some future date, to engage in a cooperative research project or program using the Material, a formal Cooperative Research and Development Agreement, or other research Agreement, must be negotiated and entered into between the parties. Such an Agreement shall supersede this Material Transfer Agreement.
13. This Material Transfer Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.

This Material Transfer Agreement shall become effective upon date of final signature and shall continue in effect for a period of [state a period of one to five (1-5) years].

**ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:**

QA#:	Permit Information (Type and Number):	
Typed name/Title	Signature (NWRC APHIS Scientist)	Date
Typed Name/Title	Signature (NWRC APHIS Assistant Director)	Date

**ACCEPTED FOR RECIPIENT:**

Typed Name/Title	Signature	Date
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**APPROVED:**

Typed Name/Title	Signature (Technology Transfer Coordinator)	Date
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Original: NWRC Agreements Specialist  
cc: Technology Transfer Program Manager, Quality Assurance Unit

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**Material Transfer Agreement**

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**ANIMAL / ANIMAL TISSUE TRANSFER AGREEMENT  
U. S. Department of Agriculture  
Animal and Plant Health Inspection Service  
National Wildlife Research Center****PARTIES:**

**APHIS:** USDA, APHIS  
National Wildlife Research Center  
Scientist Address  
City, State Zip  
Tel: Telephone # of Scientist  
FAX: FAX # of Scientist  
E-Mail: E-mail address of Scientist

**Recipient:** Company Name  
Company Address  
City, State Zip of Company  
Tel: Telephone # of Recipient  
FAX: FAX # of Recipient  
E-mail: E-mail address of Recipient

**PURPOSE:**

To provide Recipient with the following animals, animal tissues, or biological samples, hereinafter collectively known as the Material:

*[Table may be adjusted as needed]*

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material and shall provide APHIS with any manuscript that describes the work with the Material and acknowledge APHIS' contribution to the work reported when appropriate.

5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Animal Welfare Act, the Center for Disease Control, and /or Export Control Administration and all federal and state wildlife regulations pertaining to possession, transport or transference of animals, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, and the like.
7. Upon completion of the activities performed using the Material, the Material shall be [redacted] [for example, returned to ..., destroyed by..., disposed of as instructed by APHIS].
8. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. FURTHERMORE, APHIS GIVES NO WARRANTIES THE MATERIAL IS FREE OF PATHOGENS OR DISEASE. *[Add this or similar option when there is reasonable belief all or some of the material may be contaminated]* THIS MATERIAL MAY BE INFECTED WITH PATHOGENS *[be specific when warranted]*. RECIPIENT AGREES TO USE MATERIALS IN ACCORDANCE WITH LOCAL, STATE AND FEDERAL LAWS GOVERNING THE USE AND DISPOSAL OF THESE PATHOGENS.
9. This Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia.
10. *[Delete if not needed]* Other Conditions/Considerations: [redacted]

This Agreement shall become effective upon date of final signature and shall continue in effect until all Material is appropriately returned or disposed.

#### ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

QA#:	Permit Information (Type and Number):	
<hr/>	<hr/>	<hr/>
Typed name/Title	Signature (NWRC APHIS Scientist)	Date
<hr/>	<hr/>	<hr/>
Typed Name/Title	Signature (NWRC APHIS Project Leader)	Date

#### ACCEPTED FOR RECIPIENT:

<hr/>	<hr/>	<hr/>
Typed Name/Title	Signature (Technology Transfer Coordinator)	Date

Original: Quality Assurance Unit

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### Microbiological/Biohazardous Materials Use Appendix

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NWRC proposed research or testing activities which involve the use of microbiological organisms or biohazardous agents at or above a Biosafety Level 2 or Risk Level 2, or use recombinant DNA *in vivo*, require this appendix to be completed and submitted to the NWRC IBC for review and approval.

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at [www.cdc.gov/od/ohs/biosfty/biosfty.htm](http://www.cdc.gov/od/ohs/biosfty/biosfty.htm) for the definitions and lists of BioSafety Level 2 organisms and above.

Reference the American Biological Safety Association's (ABSA) "Risk Group Classification for Infectious Agents" at <http://www.absa.org/resriskgroup.html> for the definitions and lists of Risk Level 2 agents and above.

Reference the National Institute of Health's (NIH) Guidelines for Recombinant DNA and Gene Transfer at [www4.od.nih.gov/oba/rac/documents1.htm](http://www4.od.nih.gov/oba/rac/documents1.htm) for specific practices for constructing and handling recombinant DNA and organisms/viruses containing recombinant DNA molecules. Definition of recombinant DNA; 1) Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or 2) Molecules that result from the replication of those in 1 above.

**A. Identify the organism(s)/agent to be used (e.g., species, strain, type, etc.):**

**B. Is this a Select Agent (see [www.selectagents.gov/agentToxinList.htm](http://www.selectagents.gov/agentToxinList.htm))?**

**C. Does the organism contain recombinant DNA, or will recombinant DNA be constructed *in vivo* as a biologically active polynucleotide or polypeptide product? If yes, then address each of the following (if no, then N/A):**

1. The source(s) of the DNA.
2. The nature of the inserted DNA sequences.
3. The host(s) and vector(s) to be used.
4. Will an attempt be made to obtain expression of a foreign gene? If so, indicate the protein that will be produced.
5. The containment conditions that will be implemented.

**D. Source of the organism(s)/agent (e.g., location or name and address of lab/vendor):**

**E. Procedures for shipping and transportation (e.g., from facility to facility, and from room to room):**

**F. Location(s) where the materials are to be used and stored (include all buildings and room number and laboratories):**

**G. Permit information:**

**H. Inventory and tracking procedures (e.g., chain of custody procedures):**

**I. Quality control measures (e.g., procedures to prevent contamination of stocks):**

**Agent Hazards:**

**J. What particular hazards to humans, animals, and the environment are associated with these organisms/agents?** (e.g., infective dose, severity of disease, mode of transmission, susceptibility to humans, stability in the environment, etc.)

**Laboratory Procedure Hazards:**

**K. Estimated volume, amount or concentration of agents or solutions:**

**L. Identify known or potential sources of contamination or exposure** (e.g., infected live animals, tissues, fluids, byproducts, waste, sharps, etc.)

**M. Identify any procedures and equipment which could produce aerosols** (e.g., pipetting, blenders, centrifuges, sonication and vortexing), and describe how the creation of aerosols and/or exposures to those aerosols will be minimized.

**Biosafety, Security and Additional Precautions:**

**N. Biosafety Level / Risk Level (from the CDC or ABSA reference above):**

**O. Biosecurity Plan** (the Biosecurity Plan is a description of a number of different aspects which together define the mechanisms by which biohazardous agents will be safely and securely used)

**1. Physical Security:** Describe procedures to prevent unauthorized access or use of the organisms/materials.

**2. Biosecurity:** Describe the procedures, processes, facility controls and equipment that will be used to ensure biosecurity. Include but not limited to: Description of containment; Bio-inclusion (procedures to keep biological agents in containment); Bio-exclusion (procedures to keep unwanted biological agents out of containment); Decontamination (including work surfaces, materials, cages, equipment, rooms, etc.); and Disposal procedures, including carcass disposal.

**P. Specialized Risk Control Measures:**

Describe specialized risk control measures to be used to protect personnel and prevent exposures. Describe items that are specific or unique for this study (e.g., personal protective equipment, immunizations or medical surveillance, training, or other specialized precautions, equipment, or practices).

**T. Provide an assurance statement that all practices and procedures are in accordance with the appropriate guidelines for that biosafety/risk level of organism/materials:**

**U. NWRC Institutional Biosafety Committee (IBC):**

Date of IBC approval letter: \_\_\_\_\_

## NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

- ☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.
- ☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment
- ☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:
- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
  - ☐ B) not cause contaminants to enter water bodies
  - ☐ C) not adversely affect any federally protected species or critical habitat
  - ☐ D) not cause bioaccumulation
- ☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

- ☐ No
- ☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

**Effects on T&E species and eagles:**

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☐ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

**Consultations:**

Did you consult with a state or federal agency specifically on this action.

☐ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☐ Yes

## Test, Control and Reference Material/Devices Formulation and Use Appendix

### A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code
  - a) Concentration and purity:
  - b) Source:
  - c) Batch number:

For non-standard materials, describe the material/device in detail and provide the name and location of the formulation laboratory or facility that will prepare the material.

### B. Describe any control or reference materials/devices

As above, for each material provide the chemical, bait or device

- 1) name or code
  - a) Concentration and purity:
  - b) Source:
  - c) Batch number:

### C. Carriers, mixtures and material preparation

Give a full description of any carriers for the test/reference substance, mixing procedures, bait formulation procedures and a full description of possible contaminants and acceptable ranges for them. Include solvents, emulsifiers, dietary/bait materials and/or other materials used to dissolve or suspend the test or control substances.

If materials are to be prepared by NWRC TCRS Custodian complete the following:

TCRS Custodian Consultation: \_\_\_\_\_ Date: \_\_\_\_\_

### D. Route of administration

Describe the route of administration of the test substance and give a reason for its selection.

### E. Dosage

Define the dose levels of the test or control substances in appropriate units of measurement, and the frequency of administration.

### F. Test, control, and reference substance accountability

Cite the appropriate SOP(s) (e.g., AD 012) for substance accountability or describe how these materials will be appropriately documented, handled, tracked and disposed of. For all TCRSs to be used in a regulated or potentially regulated study, for which NWRC characterization is required, or when required by the Study Director or Sponsor, a retention sample must be taken and provided to the Analytical Chemistry Project for archive. For studies meeting these requirements, indicate the TCRS tracking number below.

TRCS tracking number(s): \_\_\_\_\_



**G. Material verification**

Include how and when the test material will be sampled and tested for identity, strength, purity, stability and uniformity, as appropriate.

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

ACP Consultation: \_\_\_\_\_ Date: \_\_\_\_\_

## PROTOCOL AMENDMENT / CHANGE / REVISION

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Study Director: Jack C. Rhyan Amendment No.: 1 Page 1 of 1

Study title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

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### Changes in schedule:

<input checked="" type="checkbox"/>	No schedule changes		
<input type="checkbox"/>	Experiment Start Date:	(current) _____	(revised) _____
<input type="checkbox"/>	Experiment Termination Date:	(current) _____	(revised) _____
<input type="checkbox"/>	Study Completion/Archive Date:	(current) _____	(revised) _____

### Protocol section/subsection/appendix to be changed:

Section 8. Methods/Procedures

### Description of revisions: *(Please provide the level of detail normally required in the protocol)*

The statement:

"In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events."

Will be changed to:

"In January/February of each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events. In the first year and potentially subsequent years, all pregnant vaccinated cows will be removed and transferred to a separate paddock." These animals will be monitored in the same way as described below."

### Justification/reason(s) for changes and impact on study: *(If dates are changed, please provide a description of current status of study and remaining study plan/schedule.)*

The objectives of this study are to assess the effects of infertility by immunocontraception on shedding and colonization of *Brucella* in infected cows. Although seronegative sentinels are present in each pen, this study is not investigating transmission of the disease; however, if pregnant vaccinates are removed from the group, there is an opportunity to monitor for presence of another infection route via the sentinels. This is not part of the objectives

---

Study Director: \_\_\_\_\_ Date \_\_\_\_\_

Project Leader: \_\_\_\_\_ Date \_\_\_\_\_

Assistant Director: \_\_\_\_\_ Date \_\_\_\_\_

NWRC IACUC / IBC (as needed): \_\_\_\_\_ Date \_\_\_\_\_

QAU received: \_\_\_\_\_ QAU reviewed: \_\_\_\_\_

Note: Sponsor approval is needed for all non-NWRC sponsored research

**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

**INTERAGENCY AGREEMENT**  
**between the**  
**ANIMAL AND PLANT HEALTH INSPECTION SERVICE**  
**and the**  
**NATIONAL PARK SERVICE**

**ARTICLE I. BACKGROUND AND OBJECTIVES**

To evaluate sterilization by use of GonaCon™, an immunocontraceptive vaccine, and ovariectomy as means of decreasing the potential for transmission of *Brucella abortus* in bison. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

**ARTICLE II. STATEMENT OF WORK**

A. During the period of performance, up to 63 live bison (8-16 seronegative bulls, 32-40 seropositive cows, 5-7 seronegative cows) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to determine whether:

- Immunocontraception and/or ovariectomy procedures can prevent the shedding of *Brucella abortus* bacteria in young, recently infected bison;
- Immunocontraception with GonaCon™ vaccine can prevent shedding of *Brucella abortus* bacteria throughout the infection cycle;
- Recovery from the contraceptive treatment and the brucellosis infection can be completed without any further shedding of the bacteria during subsequent pregnancies; and
- Behavioral changes occur during the breeding season when females are treated with two types of pregnancy prevention procedures.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

D. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

**ARTICLE III. TERM OF AGREEMENT**

The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

**ARTICLE IV. KEY OFFICIALS**

National Park Service  
Yellowstone Center for Resources  
Rick Wallen, Wildlife Biologist

Animal and Plant Health Inspection Service  
Veterinary Services  
Jack Rhyan, DVM

P.O. Box 168  
Yellowstone National Park, WY 82190  
307-344-2285  
rick\_wallen@nps.gov

National Wildlife Research Center  
Fort Collins, CO 80521  
970-266-6140  
Jack.C.Rhyan@aphis.usda.gov

## **ARTICLE V. PAYMENT**

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

## **ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION**

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status. Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure should be consigned to a quarantine location for further diagnostics, directly to a managed for public trust conservation program to supplement population genetic diversity, to an introduction program to establish a new conservation population of wild bison, or if no such opportunities exist, to a private not-for-profit bison conservation program. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis-free bison to any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.



## ARTICLE VII. PRIOR APPROVAL

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq., 16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in the same research study at the same location.

## ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

## ARTICLE IX. TERMINATION

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## ARTICLE X. AUTHORIZING SIGNATURES

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: \_\_\_\_\_

Name: Daniel N. Wenk

Title: Superintendent, Yellowstone NP

Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: ??????????

Title: ??????????

Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: Tina Holland

Title: Contracting Officer

Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: ??????????

Title: ??????????

Date: February \_\_\_\_, 2013

**INTERAGENCY AGREEMENT**  
**between the**  
**ANIMAL AND PLANT HEALTH INSPECTION SERVICE**  
**and the**  
**NATIONAL PARK SERVICE**

**ARTICLE I. BACKGROUND AND OBJECTIVES**

To evaluate the use of assisted reproduction techniques as means of genetic preservation for bison that are infected with *Brucella abortus*. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

**ARTICLE II. STATEMENT OF WORK**

A. During the period of performance, up to 15 live bison (12 adult cows and 3 adult bulls) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to evaluate embryos, offspring, and recipients for transmission of brucellosis via embryo transfer when in vivo and in vitro produced embryos are generated from cows and bulls with various titers of *Brucella abortus*. The rationale for this experiment is proof of principle that brucellosis-free embryos can be generated using oocytes and semen from infected animals without transmission of disease to embryo recipients or offspring.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

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Rick Wallen, Wildlife Biologist  
P.O. Box 168  
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Jack Rhyan, DVM  
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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.

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C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

## **ARTICLE VII. PRIOR APPROVAL**

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq., 16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in the same research study at the same location.

#### **ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES**

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

#### **ARTICLE IX. TERMINATION**

Either party may terminate the agreement by providing 14 days advance written notice to the other party.

#### **ARTICLE X. AUTHORIZING SIGNATURES**

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: \_\_\_\_\_

Name: Daniel N. Wenk

Title: Superintendent, Yellowstone NP

Date: February \_\_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: ??????????

Title: ??????????

Date: February \_\_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: Tina Holland

Title: Contracting Officer

Date: February \_\_\_\_\_, 2013

Signature: \_\_\_\_\_

Name: ??????????

Title: ??????????

Date: February \_\_\_\_\_, 2013

**INTERAGENCY AGREEMENT**  
**between the**  
**ANIMAL AND PLANT HEALTH INSPECTION SERVICE**  
**and the**  
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**ARTICLE I. BACKGROUND AND OBJECTIVES**

To evaluate the use of assisted reproduction techniques as means of genetic preservation for bison that are infected with *Brucella abortus*. This agreement is between the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and the U.S. Department of Interior, National Park Service, Yellowstone National Park.

**ARTICLE II. STATEMENT OF WORK**

A. During the period of performance, up to 15 live bison (12 adult cows and 3 adult bulls) may be transferred by the National Park Service from the Stephens Creek capture facility in Yellowstone National Park. The animals will be held by the Animal and Plant Health Inspection Service in fenced quarantine pastures in Corwin Springs, Montana prior to being transferred for use in a reproductive study at an affiliated research location (National Wildlife Research Center in Ft Collins, CO). The Animal and Plant Health Inspection Service will conduct an experimental research study with these bison to evaluate embryos, offspring, and recipients for transmission of brucellosis via embryo transfer when in vivo and in vitro produced embryos are generated from cows and bulls with various titers of *Brucella abortus*. The rationale for this experiment is proof of principle that brucellosis-free embryos can be generated using oocytes and semen from infected animals without transmission of disease to embryo recipients or offspring.

B. Additional Yellowstone bison may be transferred by the National Park Service to the Animal and Plant Health Inspection Service for this research study in subsequent years based on written bilateral modification of this agreement.

C. All data collected by the Animal and Plant Health Inspection Service during this research study will be provided to the National Park Service in the form of data releases and/or interim and final reports.

D. Changes to this agreement may be affected by issuance of a written modification hereto which both parties execute.

**ARTICLE III. TERM OF AGREEMENT**

The period of performance of this agreement will be from February 1, 2013, through January 31, 2017 at which time both parties will review and evaluate the agreement for possible extension.

**ARTICLE IV. KEY OFFICIALS**

National Park Service  
Yellowstone Center for Resources  
Rick Wallen, Wildlife Biologist  
P.O. Box 168  
Yellowstone National Park, WY 82190  
307-344-2285

Animal and Plant Health Inspection Service  
Veterinary Services  
Jack Rhyan, DVM  
National Wildlife Research Center  
Fort Collins, CO 80521  
970-266-6140

## **ARTICLE V. PAYMENT**

A. The National Park Service will not charge the Animal and Plant Health Inspection Service a fee for the bison that are provided to it. The National Park Service cannot guarantee a specific number of bison to the Animal and Plant Health Inspection Service in any given year.

B. The National Park Service and the Animal and Plant Health Inspection Service will use their own respective funding sources to accomplish their respective tasks. The National Park Service will not pay for or provide equipment, funding, or personnel for bison transport or security to the Animal and Plant Health Inspection Service, or vice versa.

C. This agreement may be renewed yearly if agreeable to both parties. Renewals shall be in the form of a written bilateral modification. It is mutually understood that renewals are subject to the availability of funds for future work; and it is hereby agreed that, if funds are not available, the Animal and Plant Health Inspection Service shall release the National Park Service from any liabilities and future commitment under this agreement.

## **ARTICLE VI. PROPERTY MANAGEMENT AND DISPOSITION**

A. The Animal and Plant Health Inspection Service will assume ownership of the bison in Yellowstone National Park once they are loaded, secured, and manifested into trailers or other vehicles appropriate for transporting bison.

B. When any Yellowstone bison are no longer needed for the purposes of the research experiment described in Article II, Statement of Work, they should be consigned based on their brucellosis status. Bison that test positive for brucellosis exposure should be consigned to a terminal pasture, an educational display, or if no such options are available, then directly to a slaughter facility. Bison that test negative for brucellosis exposure should be consigned to a quarantine location for further diagnostics, directly to a managed for public trust conservation program to supplement population genetic diversity, to an introduction program to establish a new conservation population of wild bison, or if no such opportunities exist, to a private not-for-profit bison conservation program. If none of these opportunities can be accommodated, then a last choice would be to offer brucellosis-free bison to any private party that requests transfer of ownership.

C. Pursuant to 36 CFR part 10, Yellowstone bison transferred to individuals and private institutions cannot be slaughtered or released without adequate protection from premature hunting. If no feasible or suitable parties agree to receive the bison and obtain all the necessary agreements to implement this action, then the bison may be consigned to slaughter facilities (with meat and other body parts distributed to tribes and food banks) or vaccinated and returned to the Yellowstone bison population.

D. The Animal and Plant Health Inspection Service agrees that the live Yellowstone bison in the experimental research study described in this agreement are to be used solely for research purposes, are to be used only at the organization's facilities for this research and only under the direction of their Key Official for this agreement or others working under his supervision, and will not be transferred to anyone else without notification of Yellowstone National Park.

## **ARTICLE VII. PRIOR APPROVAL**

The National Park Service authorities for entering into this agreement are 16 U.S.C. § 1 et seq.,

16 U.S.C. § 3, and 16 U.S.C § 36.

During 2011, the National Park Service transferred 52 bison (4 males, 48 females) from the Stephens Creek capture facility in Yellowstone National Park to the Animal and Plant Health Inspection Service for transport to fenced quarantine pastures in Corwin Springs, Montana. The Animal and Plant Health Inspection Service began conducting an experimental research study with these bison as described in Article II, Statement of Work. This agreement allows additional bison to be transferred for use in a reproductive study at an affiliated research location (National Wildlife Research Center in Ft Collins, CO).

#### **ARTICLE VIII. REPORTS AND/OR OTHER DELIVERABLES**

The Animal and Plant Health Inspection Service shall provide annual and final reports to the Key Official for the National Park Service on this agreement for all data collected during this study.

#### **ARTICLE IX. TERMINATION**

Either party may terminate the agreement by providing 14 days advance written notice to the other party.

#### **ARTICLE X. AUTHORIZING SIGNATURES**

IN WITNESS HEREOF, the parties hereto have signed their names and executed this Interagency Agreement.

National Park Service:

Animal and Plant Health Inspection Service:

Signature: \_\_\_\_\_  
Name: Daniel N. Wenk  
Title: Superintendent, Yellowstone NP  
Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_  
Name: Jack Rhyan  
Title: APHIS Veterinary Officer  
Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_  
Name: Tina Holland  
Title: Contracting Officer  
Date: February \_\_\_\_, 2013

Signature: \_\_\_\_\_  
Name:  
Title:  
Date: February \_\_\_\_, 2013



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

[bfc-media@wildrockies.org](mailto: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](mailto:bfc-media@wildrockies.org) as well as on a CD, so as to reduce time, cost and waste.* Disclosure of the documents I requested is in the public interest because it is likely to contribute significantly to public understanding of the operations or activities of the government and is not primarily in my commercial interest.

Sincerely,

  
Stephany J. Seay  
Buffalo Field Campaign

Cc:

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

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

[bfc-media@wildrockies.org](mailto: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,

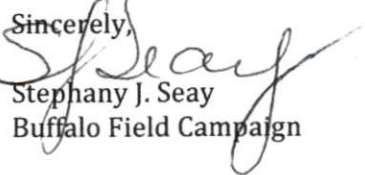
On behalf of Buffalo Field Campaign, a Montana-based wild bison advocacy group representing tens of thousands of concerned citizens in Montana, throughout the United States and around the globe, working in defense of America's last wild bison population, the Yellowstone herds, I file a Freedom of Information 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](mailto:bfc-media@wildrockies.org) as well as on a CD, so as to reduce time, cost and waste.*

Sincerely,

  
Stephany J. Seay  
Buffalo Field Campaign

Cc:

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

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

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

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

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

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

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

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

**USAHA Committee on Wildlife Diseases**  
**November 16, 2010, 8:00AM – 12:00 PM**  
**Salon B**  
**Minneapolis Hilton**  
**Minneapolis, Minnesota**

**Agenda**

Dr. Stephen M. Schmitt, Chair and Dr. Colin M. Gillin, Co-Chair

**Bighorn Sheep**

8:00-8:05	Introductory Comments	Steve Schmitt, Colin Gillin
8:05-8:25	Report of the Wild/Domestic Sheep Working Group	Walt Cook
8:25-8:50	Pneumonia in Bighorn Sheep	Sri Subramaniam

**Cervids and Bison**

8:50-9:05	Bovine Tuberculosis in Minnesota Wildlife	Erika Butler
9:05-9:20	CWD National Program	Pat Klein
9:20-9:40	Brucellosis Transmission Dynamics in the Northern GYA	Brant Schumaker
9:40-9:55	Brucellosis Challenges in GYA	Marty Zaluski
9:55-10:10	Brucellosis in Wildlife in the GYA	Mark Drew
10:10-10:25	Hemorrhagic Disease	Mark Ruder

**Wolves, Bats, Wild Birds and Prairie Dogs**

10:25-10:40	Echinococcus in Wolves	Mark Drew
10:40-11:00	White-Nose Syndrome in Bats	David Blehert
11:00-11:15	Avian Influenza Research	Justin Brown
11:15-11:30	Oral Plaque Vaccine	Tonie Rocke

**Committee Business**

11:30-12:00	Resolutions and Other Committee Business	Steve Schmitt, Colin Gillin
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## BioTracking LLC

1150 Alturas Dr  
Suite 105  
Moscow, ID 83843  
Phone: 208.882.9736  
Fax: 208.882.1490  
email: [biotracking@biotracking.com](mailto:biotracking@biotracking.com)  
web: [www.biotracking.com](http://www.biotracking.com)

## BioPRYN PSPB Report

Date Received	Log In #
2/4/2014	O20414001

### Submitted By

USDA/APHIS/VS  
4101 LaPorte Avenue  
Fort Collins, CO 80521

### Report To

Dr. Jack Rhyan

[jack.c.rhyan@aphis.usda.gov](mailto:jack.c.rhyan@aphis.usda.gov)

### REPORT NOTES:

Mail Report

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

Report Date	Assay/Animal	Number of Samples
02/05/2014	Bison - 52 sample(s)	52

Open	Low Recheck	Cutoff	High Recheck	Pregnant
OD < 0.135	OD = 0.135 to 0.15	0.15	OD = 0.15 to 0.21	OD > 0.21

Tube Number	Animal ID	Response in Test, OD	PSPB Range	Days Post Breeding
1	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
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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## 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



**Proposed Project:**

**DRAFT**

**Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.**

**Investigators:**

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

**Background:**

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy thereby preventing parturition or abortion 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.

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#### 3.4. Provide brucella negative animals to conservation herds.

##### **Research Plan:**

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the ~~facilities~~ facilities. These facilities comprise ~~7~~ 5 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

**Pasture A** will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

**Pasture B** will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December until July. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements ~~as~~ published in the UM&R will be used for bison conservation. (The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.) Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at- minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

**Time line:**

**Winter/spring 2011** – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

**Spring 2012** – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

**Winter/Spring 2013-2015** – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December ~~til~~ through July each year.

**Summer 2015** – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults ~~and~~ offspring meet requirements of quarantine, use for bison conservation.

**Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

5.6. Animals that pass the quarantine requirements will be incorporated into conservation herds.

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Accession	Casa #	Submitter	S. C. Ty.	S. State	Owner	O. C. Ty.	Owner State	Animal Country	Animal Strain	Species	Sample ID	Animal ID	Identif cat on	Year Received	ID	State	County Code	Species	He d Year	He d Letter	Animal Label	Master Po e Date	Regl cat on	Public (Y/N)	PATRIC	Source	AT	SNP Val dated	Genotyping code		
602350	B09-0553	Cla ke, D. Ryan	Belg ade	MT	BQFS	Co w n Sp ngs	MT	Pa k	MT	B son		85-08	B ucella abou tus b ova 1	09		BA1	MT	067	Bi	WL	x	1		N	N	Sent	Y		B09-0553_09BA1_MIT-067_Bi-WLx1		
10-023707	B10-0973	Cla ke, P. Ryan-bull b son study	Belg ade	MT	USDA/APHIS/V5	Belg ade	MT	Gallat n	MT	B son		YNP95021	B ucella abou tus b ova 1	10		BA1	MT	031	Bi	WL	x	8	2/24/2013	N	N		m d f		B10-0973_10BA1_MIT-031_Bi-WLx		
10-023707	B10-0975	Cla ke, P. Ryan-bull b son study	Belg ade	MT	USDA/APHIS/V5	Belg ade	MT	Gallat n	MT	B son		YNP95023	B ucella abou tus b ova 1	10		BA1	MT	031	Bi	WL	x	8	2/24/2013	N	N	Sent	Y		B10-0975_10BA1_MIT-031_Bi-WLx		
11-038123	B11-0442	MT Dept. of L vestock	Bozeman	MT	YNP	Mammoth	WY	X	MT	B son		8-456 / P11-016	B ucella abou tus b ova 1	11		BA1	MT	XXX	Bi	WL	x		1/30/2013	N	N	Sent	Y		B11-0442_11BA1_MIT-XXX_Bi-WLx		
11-019422	B11-0469	MT Dept. of L vestock	Bozeman	MT	YNP	Mammoth	WY	X	WY	B son		8-458 / P11-017	B ucella abou tus b ova 1	11		BA1	MT	XXX	Bi	WL	x		1/14/2013	N	N	Sent	Y		B11-0469_11BA1_MIT-XXX_Bi-WLx		
11-020659	B11-0480	Cla ke, D. Ryan	Belg ade	MT	Yellowstone Nat onal Pa k w ld fe	West Ye lowstone	MT	Gallat n	MT	B son		YNP950043	B ucella abou tus b ova 1	11		BA1	MT	031	Bi	WL	x		1/14/2013	N	N	Sent	Y		B11-0480_11BA1_MIT-031_Bi-WLx		
11-021534	B11-0488	MT Dept. of L vestock	Bozeman	MT	YNP	Mammoth	WY	X	WY	B son		8-470 / P11-024	B ucella abou tus b ova 1	11		BA1	MT	XXX	Bi	WL	x		1/14/2013	N	N	Sent	Y		B11-0488_11BA1_MIT-XXX_Bi-WLx		
11-040274	B11-0574	Rhyon, D. Jack	Fo t Coll ns	CO	USDA	Fo t Coll ns	CO			B son		711	B ucella abou tus b ova 1	11		BA1			Bi	WL	x		1/14/2013	N	N		Y		B11-0574_11BA1_Bi-WLx		
12-030298	B12-0460	Rhyon, D. Jack	Fo t Coll ns	CO	USDA/APHIS/V5	Fo t Coll ns	CO			B son		017	B ucella abou tus b ova 1	12		BA1			Bi	WL	x	1/3/2013		N	N	Sent	Y		B12-0460_12BA1_Bi-WLx		
12-034627	B12-0560	Rhyon, D. Jack	Fo t Coll ns	CO	USDA/APHIS/V5	Fo t Coll ns	CO			B son		017	B ucella abou tus b ova 1	12		BA1			Bi	WL	x	1/3/2013		N	N	Sent	Y		B12-0560_12BA1_Bi-WLx		
13-005136	13-0061	Cla ke, 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 abou tus b ova 1	13		BA1	MT	67	Bi	WL	x		B	2/24/2013		N	N	Sent	Y		B13-0061_13BA1_MIT-067_Bi-WLx
559510	08-0783	Cla ke, D. Ryan	Belg ade	MT	USDA/APHIS/V5 / BQFS	Bozeman	MT	Pa k	MT	B son		9-08 / B1ARG3891	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-0783_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
559510	08-0795	Cla ke, D. Ryan	Belg ade	MT	USDA/APHIS/V5 / BQFS	Bozeman	MT	Pa k	MT	B son		1-08 / B1AYE4039	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-0795_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
561953	08-0931	Rhyon, D. Jack	Fo t Coll ns	CO	Rhyon, D. Jack (Nat l W ld fe Resea ch Cnt )	Fo t Coll ns	CO	Pa k	MT	B son		9-08	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-0931_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
561953	08-0933	Rhyon, D. Jack	Fo t Coll ns	CO	Rhyon, D. Jack (Nat l W ld fe Resea ch Cnt )	Fo t Coll ns	CO	Pa k	MT	B son		1-08	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-0933_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
563110	08-1019	Cla ke, D. Ryan	Belg ade	MT	USDA/APHIS/V5/BQFS	Bozeman	MT	Pa k	MT	B son		0-08 / B1ARG3889	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-1019_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
563110	08-1080	Cla ke, D. Ryan	Belg ade	MT	USDA/APHIS/V5/BQFS	Bozeman	MT	Pa k	MT	B son		1-08 / B1APM1605	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-1080_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
563912	08-1122	Cla ke, D. Ryan	Belg ade	MT	BQFS	Co w n Sp ngs	MT	Pa k	MT	B son		1-08 / B1APM1605	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-1122_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
563912	08-1124	Cla ke, D. Ryan	Belg ade	MT	BQFS	Co w n Sp ngs	MT	Pa k	MT	B son		0-08 / B1ARG3889	B ucella abou tus b ova 1	08		BA1	MT	067	Bi	WL	x			N	N		Y		B08-1124_08BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
13-013440	13-0083	Cla ke, D. Pat ck R.	Belg ade	MT	USDA/APHIS/V5-BQ-Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 16	Red 16	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	4/25/2013 5-1 Plac		N	N		Y		B13-0083_13BA1_MIT-067_Bi-WLx	
13-013849	13-0086	MT Dept. of L vestock	Bozeman	MT	USDA/APHIS/V5-BQ-Gonacon	Ga d ne	MT	Pa k	MT	B son	8-435	Red 16 fetus / b son	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	MT		N	N		Y		B13-0086_13BA1_MIT-067_Bi-WLx	Stephens C eek T ap	
13-015343	13-0090	Cla ke, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	5/9/2013 5-1 Vagfx, 5-2 Vagfsw, 5-3 Plac5w		N	N		Y		B13-0090_13BA1_MIT-067_Bi-WLx	
13-015343	13-009052	Cla ke, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	5/9/2013 5-1 Vagfx, 5-2 Vagfsw, 5-3 Plac5w		N	N		Y		B13-009052_13BA1_MIT-067_Bi-WLx	
13-015343	13-009053	Cla ke, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	Red 21	Red 21	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	5/9/2013 5-1 Vagfx, 5-2 Vagfsw, 5-3 Plac5w		N	N		Y		B13-009053_13BA1_MIT-067_Bi-WLx	
13-015343	13-0091	Cla ke, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	G een 09	G een 09	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	5/9/2013 5-1 Plac, 5-2 M lnc		N	N		Y		B13-0091_13BA1_MIT-067_Bi-WLx	
13-015343	13-009152	Cla ke, D. Ryan	Belg ade	MT	B son Qua ant ne Gonacon	Co w n Sp ngs	MT	Pa k	MT	B son	G een 09	G een 09	B ucella abou tus b ova 1	13		BA1	MT	067	Bi	WL	x	8	5/9/2013 5-1 Plac, 5-2 M lnc		N	N		Y		B13-009152_13BA1_MIT-067_Bi-WLx	

## 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/](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](mailto: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

Bison Work  
5 August, 2015

Animal ID	Weight	Sex	Ivermectin (ml)	Ear Tags	Blood	Feces	Vaccinations	Comments

Ivermectin Dose 1cc/110#

Ear Tags: Python

Blood: 1 Tiger Top and 1 Green Top

Feces: Johne's PCR +/- Culture

Vaccinations: Rabies, 9-Way, Cattle Master FP5, Calf Guard

Bison Work  
August, 2015  
Date:

Animal ID	Weight	Sex	Ivermectin (ml)	Ear Tags	Blood	Feces	Vaccinations	Comments

Ivermectin Dose 1cc/110#  
Ear Tags: Python Magnum  
Blood: 1 Tiger Top and 1 Green Top  
Feces: Johne's PCR +/- Culture  
Vaccinations: Rabies, 9-Way, Cattle Master FP5, Calf Guard





## 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**

Jack Rhyan  
Fort Collins, CO

**Animal Location**

Larimer County CO

**Submitter - 2649**

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

**Accession Number:**

**15-017999**

**Date Collected:****Date Received:**

06/04/2015

**Date Completed:****Collected By:**

06/25/2015

Jack Rhyan

**Purpose:**

NVSL Internal

**Referral Number:****Country Origin/Destination:**

**This is not a billable case.**

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

**Sample: 1 Specimen Type: Serum Animal ID: 4R21 (5/28/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	0 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 2 Specimen Type: Serum Animal ID: 4R16 (5/28/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	2 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 3 Specimen Type: Serum Animal ID: 420 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	3 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 4 Specimen Type: Serum Animal ID: 62 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-2 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	QNS
Brucellosis (Brucella abortus/suis) - Plate Test	QNS
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 5 Specimen Type: Serum Animal ID: 69 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	3 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	QNS
Brucellosis (Brucella abortus/suis) - Plate Test	QNS
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 6 Specimen Type: Serum Animal ID: 3R21 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	6 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Incomplete Agglutination@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 7 Specimen Type: Serum Animal ID: 66 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	0 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Incomplete Agglutination@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 8 Specimen Type: Serum Animal ID: 63 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative 0@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-4 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 9 Specimen Type: Serum Animal ID: 5R18 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-81 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 10 Specimen Type: Serum Animal ID: 61 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	3 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 11 Specimen Type: Serum Animal ID: 3R13 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	1 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 12 Specimen Type: Serum Animal ID: 3R26 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 3+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	1 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 13 Specimen Type: Serum Animal ID: 65 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 2+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-5 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	<b>Positive@1:100</b>
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 14 Specimen Type: Serum Animal ID: 130 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-1 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Incomplete Agglutination@1:100
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 15 Specimen Type: Serum Animal ID: 3R30 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	1 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Incomplete Agglutination@1:50
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 16 Specimen Type: Serum Animal ID: 3R7 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	1 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Incomplete Agglutination@1:50
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 17 Specimen Type: Serum Animal ID: 3R25 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	0 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Incomplete Agglutination@1:50
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 18 Specimen Type: Serum Animal ID: 3R24 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 2+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	-3 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Incomplete Agglutination@1:50
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 19 Specimen Type: Serum Animal ID: 52R (5/6/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	<b>Positive</b>
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 1+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	91/83 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	<b>Positive@1:50</b>
Brucellosis (Brucella abortus/suis) - Plate Test	Incomplete Agglutination@1:100
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 20 Specimen Type: Serum Animal ID: 47 (5/6/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	<b>Positive</b>
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 4+@1:160</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	96/82 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	<b>Positive@1:100</b>
Brucellosis (Brucella abortus/suis) - Plate Test	Incomplete Agglutination@1:100
Brucellosis (Brucella abortus/suis) - Card Test (8%)	<b>Positive</b>

**Sample: 21 Specimen Type: Serum Animal ID: R57 (10/9/14) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	<b>Positive</b>
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 4+@1:320</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	180/173 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Incomplete Agglutination@1:200
Brucellosis (Brucella abortus/suis) - Plate Test	<b>Positive@1:100</b>
Brucellosis (Brucella abortus/suis) - Card Test (8%)	<b>Positive</b>

**Sample: 22 Specimen Type: Serum Animal ID: 156 (10/9/14) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	<b>Positive</b>
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 4+@1:320</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	154/155 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	<b>Positive@1:200</b>
Brucellosis (Brucella abortus/suis) - Plate Test	<b>Positive@1:200</b>
Brucellosis (Brucella abortus/suis) - Card Test (8%)	<b>Positive</b>

**Sample: 23 Specimen Type: Serum Animal ID: 4R7 (5/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	<b>Positive 3+@1:10</b>
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	5 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**Sample: 24 Specimen Type: Serum Animal ID: 3G02 (1/27/15) Animal Status: Species: Bison**

Brucellosis (Brucella abortus/suis) - Buffered Acidified Plate Antigen (BAPA) Test	Negative
Brucellosis (Brucella abortus/suis) - Complement Fixation (CF - Cold ) Test	Negative tr@1:10
Brucellosis (Brucella abortus/suis) - Fluorescent Polarization Assay (FPA)	0 Delta mP
Brucellosis (Brucella abortus/suis) - Rivanol Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Plate Test	Negative@1:25
Brucellosis (Brucella abortus/suis) - Card Test (8%)	Negative

**FPA:****Delta mP > 20 = Positive****Delta mP 10-20 = Suspect****Delta mP < 10 = Negative****Results authorized by:** Dr. David Kinker, Head, Serology (515-337-7563)**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.

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

Final ACUC protocol  
5/23/11

**REGULATORY CONSIDERATIONS**

Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____ National Park Service _____ _YELL-2011-SCI-5892_____ May 10, 2011_____ Permit(s) description _____ Number _____ Date _____

**DESCRIPTION OF ACTIVITIES**

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

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

Start Date: June 1, 2011

End Date: October 1, 2017

**STUDY PROTOCOL****1. Key Personnel**

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

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

## 2. Testing Facilities

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



serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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

In spring 2012, animals will be randomly selected to go into one of approximately 23 acres each. Each pasture will contain 16-18 seropositive cows and 4-6 seronegatives and 2 bulls. Two replicate test pastures will be established in spring 2013 or 2014 if not enough animals are captured by 2013. After 3-4 weeks acclimation, seropositive bison in one pasture will receive GonaCon™ vaccine (containing 3000µg in 3 ml adjuvant) delivered intramuscularly 1 ½ mls on either side of the neck. The sites of injection will be tattooed and measurements made from a standard landmark. All bison in the remaining pasture will not be vaccinated.

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture.

In addition, serology for each of the cows, bulls, and calves will be monitored three times a year. All bison will be tested by serology and culture in February, at calving time, and in the fall (September - November). Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

Specimens for culture collected during the study will be cultured immediately at NVSL, Ames,

IA.

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% ~~abortion~~ <sup>shedding</sup>). 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.

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Attn: Jack K Ryan

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Study Protocol

GonaCon-in-bison

PART ONE: SIGNATURE PAGE

Study Director:



Date:

5/16/11

Concur:  
IACUC Chair



Date

5/16/11

## CONTRACEPTION OF BISON BY GnRH VACCINE: A POSSIBLE MEANS OF DECREASING TRANSMISSION OF BRUCELLOSIS IN BISON

Lowell A. Miller,<sup>1,4</sup> Jack C. Rhyan,<sup>2</sup> and Mark Drew<sup>3</sup>

<sup>1</sup> US Department of Agriculture-Animal Plant Health Inspection Service, National Wildlife Research Center, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA

<sup>2</sup> US Department of Agriculture-Animal Plant Health Inspection Service, Veterinary Services, National Wildlife Research Center, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA

<sup>3</sup> Idaho Department of Fish and Game, 16569 S. 10th Ave., Caldwell, Idaho 83607, USA

<sup>4</sup> Corresponding author (email: lowell.a.miller@usda.gov)

**ABSTRACT:** Preventing pregnancy in brucellosis-infected bison (*Bison bison*) provides a potential means of preventing transmission of disease. To determine whether a gonadotropin-releasing hormone (GnRH) vaccine was effective in reducing pregnancy in bison and to study the safety of injecting GnRH in pregnant bison, a study was conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho (USA). Four pregnant and two nonpregnant female bison were given a single injection of GnRH vaccine, and five pregnant adult females were given a sham injection that contained only adjuvant. Three of the GnRH-vaccinated bison that were pregnant at the time of vaccination delivered healthy calves. One treated bison had dystocia that resulted in a dead calf. All control bison delivered healthy calves. After calving, females of both groups were exposed to two bulls. Treated bison were palpated 6 wk after exposure to the bulls, and blood was drawn for pregnancy-specific protein B analysis. The six treated bison were not pregnant. The sham-treated bison became pregnant and delivered viable calves. This study demonstrates that a single dose of GnRH vaccine is effective in preventing pregnancy in female bison for at least 1 yr.

**Key words:** Gonadotropin-releasing hormone, immunocontraception, GnRH vaccine, bison.

### INTRODUCTION

Bovine brucellosis, a bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*), and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or postparturient uterine discharge. Additionally, *Brucella* is shed in milk from infected dams and can be transmitted to calves through suckling. After initial infection, a dam often experiences abortion. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may also result in the shedding of *B. abortus*. The occurrence of venereal transmission of brucellosis in bison is unknown; however, on the basis of a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is considered unlikely to be a significant route of transmission. Transmission of the disease in cattle, bison, and elk, therefore, is primarily depen-

dent on the occurrence of pregnancy and exposure to abortion or calving in infected animals.

Rhyan et al. (2002) suggested that permanent sterilization, surgical or chemical, is a disease-management strategy that could be effectively used in *Brucella*-infected bison to greatly reduce the possibility of transmission to other animals. Bison cows could remain persistently infected with *B. abortus*, and, as long as the infected animals were not allowed to become pregnant, they would not be likely to transmit the infection. Therefore, disease prevalence might decrease dramatically as that generation of infected bison disappears. Objections have been raised to permanent sterilization in relation to wild horse immunocontraception, because it might result in the permanent removal of those animals from the gene pool and the creation of a new “unnatural” class of animals (Kirkpatrick and Turner, 1991).

The gonadotropin-releasing hormone

(GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns as the GnRH antibody titer drops below a protective level. This temporary period of infertility may allow time for *B. abortus* infection to clear.

The use of nonlethal methods to control populations of pest animals is an area of research that is receiving more interest (Fagerstone et al., 2002). Kirkpatrick et al. (1996) pioneered the use of porcine zona pellucida (PZP) for use as a nonlethal, contraceptive approach to pest animal control. The difficulty with the use of PZP in ungulates is that the animals that receive it, although they remain infertile, continue to have estrous cycles. Female white-tailed deer (*Odocoileus virginianus*) vaccinated with PZP have continued to exhibit sexual activity into February, 4 mo beyond the normal breeding season (Miller et al., 2000b). This continuous estrous cycling results in increased activity during early winter, at a time when the conservation of calories is important, although this increased cycling has not resulted in any apparent health problems (Miller et al., 2001). Additionally, it could increase the spread of venereally transmitted diseases, if present and, at least in the case of deer in populated areas, may contribute to increased collisions with automobiles. Prolonging the breeding season of bison in the greater Yellowstone area may be deleterious to the winter survival of dominant bulls and vaccinated cows because of increased activity during fall and early winter.

Immunocontraception using the GnRH vaccine is an alternative to PZP that would not extend the breeding season. The keyhole limpet hemocyanin–GnRH immunocontraceptive vaccine interferes with the release of follicle-stimulating hormone (FSH) and leutinizing hormone (LH), thereby preventing normal function of the ovaries and testes and their production of progesterone and testosterone. Thus, GnRH vaccine can effectively prevent

conception in either females or males (Talwar, 1985).

The GnRH vaccine has successfully produced sterility in Norway rats (*Rattus norvegicus*; Miller et al., 1997) and white-tailed deer (Miller et al., 2000a). The immunoneutralization of GnRH produces temporary nonsurgical castration in animals (Meloan et al., 1994; Oonk et al., 1998). In an ongoing study in female white-tailed deer conducted by the National Wildlife Research Center (Fort Collins, Colorado, USA) and Pennsylvania State University (University Park, Pennsylvania, USA), a single injection of GnRH vaccine resulted in infertility lasting up to 3 yr.

The development of immunocontraceptives that are practical to use for wildlife population control must include vaccine delivery systems. Although the administration of an oral form of the vaccine may be necessary in some situations, a long acting single-shot injectable form of the vaccine would have practical advantages over formulations that require two injections. Immunocontraception has typically required at least two doses, given as a prime and a boost. The prime dose prepares the immune system for a repeat antigen exposure and provides only a short-term immune response. The boost immunization can result in an immune response that may last for months to years. To have success with a single injection, the dose and timing of the injection is more critical than when using two injections. This article reports on the immunocontraception of penned bison using the newly developed single-shot GnRH vaccine.

#### MATERIALS AND METHODS

On 6 June 2002, six 6-yr-old female bison were injected with 1,800 µg of a single-shot GnRH vaccine (GonaCon/AdjuVac™, developed by the National Wildlife Research Center, United States Department of Agriculture, Animal Plant Health Inspection Service, Fort Collins, Colorado—patent pending) in a 1-ml injection given intramuscularly in the hip. Five control bison were injected with the adjuvant,

TABLE 1. Results of contraception in female bison using a GnRH vaccine.

Treatment	Year 1		Year 2		Calving dates, 2003
	Pregnancy status when injected (June 2002)	Calving dates, 2002	Pregnancy rate	PSPB <sup>a</sup> results	
Sham injection	5/5	20 June–26 July	5/5	5/5 positive for pregnancy	4 June–29 July
1,800 µg GnRH/AdjuVac <sup>b</sup>	4/6	28 June–1 July	0/6	0/6 positive for pregnancy	No calves born

<sup>a</sup> PSPB = pregnancy-specific protein B.

<sup>b</sup> GonaCon/AdjuVac, US Department of Agriculture, Animal Plant Health Inspection Service, patent pending.

1 ml, in the hip (control). All control bison and four of the treated bison were pregnant at the time of the injection. Because the GnRH vaccine has the potential to cause abortion, the pregnant bison were vaccinated to determine the safety of the GnRH vaccine. Blood samples were drawn monthly for 4 mo and then every other month for a total of 8 mo. Serum was tested for progesterone by radioimmunoassay and for GnRH antibody by enzyme-linked immunoassay (Miller et al., 2000a).

Two months after calving, a bull was introduced to the pen and allowed to breed the cows for 2.5 mo (17 September–1 December 2002). Six weeks after the bull was removed, both control and GnRH-treated bison were palpated for pregnancy diagnosis, and results were confirmed by serum pregnancy-specific protein B assay (PSPB) testing (Biotracking, Moscow, Idaho).

## RESULTS

Analysis of pregnancy and calving data in the control and GnRH-treated bison at the time of GnRH injection and the following year indicated that the GnRH vaccine was successful in reducing reproduction, compared with controls (Table 1). At

the time of vaccination, five of the sham-treated cows and three of the six GnRH-treated cows were in the last month of pregnancy. Cows in both groups delivered normal calves the first year; therefore, the GnRH vaccine did not interfere with the pregnancy. None of the GnRH-treated cows became pregnant the year after the vaccination. All control bison conceived, and four had normal calves, with calving dates of 4–30 June 2003. One control cow died on 30 March 2003 but was pregnant at the time of death. During this study, two cows, one each in the treated and control groups, had dystocia that resulted in dead calves.

The average progesterone levels for pregnant cows were the same for the treatment and control groups at the start of the study and after calving. After rebreeding, the progesterone level of cows in the control group increased to pretreatment levels, indicating that they became pregnant, and anti-GnRH titers were not detected (Fig. 1). Progesterone levels in the GnRH-treated bison remained at nonpregnant levels (Fig. 2). All control bison delivered normal healthy calves and became pregnant again the second year. One of the five control bison died from accidental causes midgestation. The remaining four controls had normal calves in year 2 of the study.

Three of the six GnRH-treated bison were in late gestation when they were immunized, and all delivered normal calves within 1 mo after treatment. Two of the GnRH-treated cows were not pregnant at the time of GnRH vaccination, as suggest-

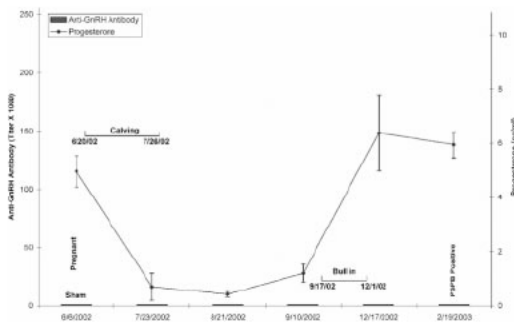


FIGURE 1. Average serum progesterone levels and anti-GnRH antibody titers for control bison cows.



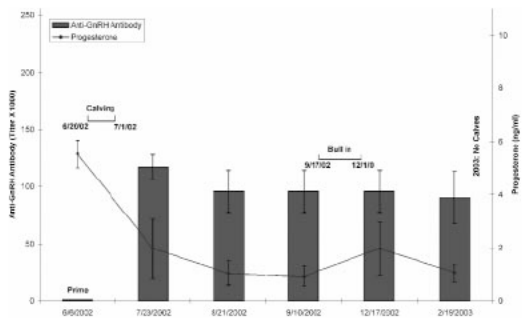


FIGURE 2. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated late during pregnancy.

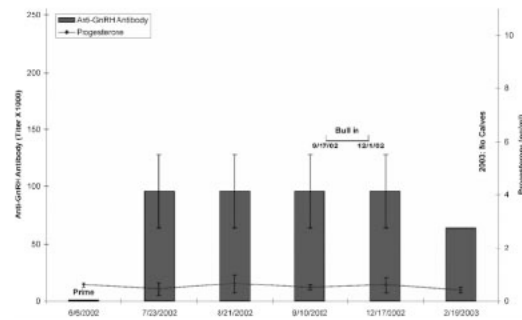


FIGURE 3. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated when not pregnant.

ed by low progesterone levels at the time of treatment (Fig. 3). The low progesterone values during the postpartum period in the cows that calved were comparable to those of the control cows. However, they did not become pregnant after exposure to the bulls, as indicated by low progesterone levels, the absence of a fetus on palpation, and negative PSPB serum test results.

An exception to the results of treatment of cows during late pregnancy was bison B-40, which had been given the GnRH vaccine during midpregnancy. This bison cow was injected with the vaccine on 6 June 2002 and delivered full term on 6 November 2002, with dystocia, resulting in a dead calf. The progesterone level progressively dropped from 7.0–3.5  $\mu\text{g}/\text{ml}$  of serum during the first 2 mo after vaccination and leveled off at 3.4  $\mu\text{g}/\text{ml}$  of serum during the third month, 2 mo before the birth of the dead full-term calf. This cow had a positive PSPB result, low progesterone levels, and was not pregnant on palpation.

The anti-GnRH data in treated bison indicated that a protective antibody titer was reached by the first time blood was collected (47 days after vaccination). The mean titer at this time was 112,000, decreasing to a mean titer of 72,000 by the end of the study. Antibody titers of 64,000 or greater have been shown to be consistent with contraception (Miller et al., 2000a).

In the beginning of the study, similar progesterone and PSPB levels in control and treated groups suggested that cows in both groups were pregnant. Calving dates were comparable in the control and treated cows, indicating synchronous breeding cycles (Table 1). However, in the second year, elevations in progesterone levels in the control group suggested that they became pregnant, and low progesterone levels in the treated group suggested infertility (Figs. 1–3).

## DISCUSSION

The GnRH vaccine induces infertility in female mammals by reducing the release of FSH and LH, which, in turn, interferes with either the normal estrous or ovulatory cycle or reduces progesterone concentration during early pregnancy, which may interfere with maintenance of pregnancy. Stevenson (1997) stated that GnRH controls the amount of progesterone produced by the corpus luteum (CL), which maintains pregnancy for 200 days of the mean 280-day gestation in cattle. After 200 days, the ovary containing the CL can be removed without interfering with pregnancy, which indicates that pregnancy is not maintained by pituitary GnRH; the placenta apparently takes over the production and maintenance of progesterone. Bison have a gestation period similar to that of cattle.

Our results indicate that the GnRH vaccine can be administered safely during the

last third of pregnancy. Protective levels of anti-GnRH antibody require 30–45 days to develop, which suggests that the vaccine could be safely administered at  $\geq 170$  days of gestation without negative effects on the fetus. This was shown to be the case in the three bison treated late in pregnancy.

One cow was vaccinated during the second trimester of pregnancy and delivered a full-term dead calf on 6 November. It is unknown whether the GnRH vaccine contributed to death the fetus. There was a decrease in progesterone levels in this cow after vaccination that could have contributed to the loss of viability of the calf. Because the bull was with the cows from 17 September to 1 December, it is unlikely that this cow could have rebred. However, anti-GnRH antibody titers were sufficient in this cow to prevent pregnancy. One control bison also had a similar late full-term dead calf; thus, it is uncertain whether the vaccine caused the death of the calf in the vaccinated cow.

All control and treated cows were tested for pregnancy by palpation and serum progesterone and PSPB levels during February 2003. Bison B-40 had a positive serum PSPB test at this time but a low progesterone level and was not pregnant on palpation. Thus, the PSPB test was incorrect. This is consistent with reports that retained placentas following abortions can cause a false-positive PSPB result for several months (Sasser et al., 1986). The bison will be monitored for 2 more years to determine the duration of the contraceptive effect.

This study demonstrates that a single injection of GnRH vaccine is effective in preventing contraception in female bison for at least 1 yr. Booster injections lengthen the contraceptive effect in white-tailed deer (Miller and Killian, 2000), and lengthening the contraceptive effect in bison may be achieved similarly. Use of the GnRH vaccine in *Brucella*-infected bison should effectively reduce transmission of disease by reducing pregnancy rates and subsequent abortion or parturition.

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*Received for publication 29 January 2004.*

# Critical Brucellosis research in MT and CO being “decommissioned” ...

Published November 23, 2017  
Category: Front Page Stories

By Leesa Zalesky

**Editor’s Note:** *Leesa and I have worked hard (she writing and me editing) to boil this comprehensive issue down to layman’s terms that I, and you the reader, can understand. This isn’t an easy story to read and comprehend as it’s beastly long, but I’d advise you first to pay close attention and then to visit with your state veterinarians for their own opinions and positions because the ramifications of the changes in Brucellosis research could very well affect us all in the cattle business. It’s high time that lawmakers and agency heads start to understand that they must listen to their constituents as well as consult with—and stop ignoring—the people closest to the ground on matters so very important. LG*

**Author’s Note:** *Brucellosis is a zoonotic disease (can be transmitted from animals to people). The genus Brucella consists of six classically recognized species based on biochemical characteristics and primary host species: Brucella abortus (cattle); Brucella melitensis (sheep and goats); Brucella suis (swine, cattle, rodents, wild ungulates; Brucella ovis (sheep); Brucella canis (dogs); and Brucella neotomae (rodents). In humans, the disease—known as undulant fever—is acquired by consuming unpasteurized milk or the under-cooked meat from an infected animal. This story generally surrounds Brucella abortus.*

LZ

## Brucellosis...

Brucellosis is an infectious, costly disease of ruminant animals that can also affect humans. Its main threat is to cattle, bison, cervids, and swine. The swath of economic damage the disease can leave behind in the cattle and dairy industries includes decreased milk production; abortion of pregnancy or the birth of weak, unhealthy calves; weight loss; infertility; and retention of afterbirth resulting in uterine infections and lameness.

Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals or with an environment that has been contaminated with discharges from infected animals. Aborted fetuses, placental membranes or fluids, and other vaginal discharges present after an infected animal has aborted or calved are all highly contaminated with infectious Brucella organisms. Cows may lick those materials or the genital area of other cows or ingest feed or water contaminated with the disease-causing organisms. The general rule is that brucellosis is carried from one herd to another by an infected or exposed animal. The disease may also be spread when wildlife or animals from an affected herd mingle with brucellosis-free herds.

## Vaccines and research...

A brucellosis vaccine is used in cattle to prevent the disease, but it is not 100% effective. The vaccine’s efficacy rate is 70 – 80% in preventing vaccinated cattle from being infected by an average exposure. While vaccination is a very important tool in brucellosis prevention, it’s easy to see that more scientific research must be done to improve the vaccines used. That same field research will help scientists understand more about how the disease develops and spreads. Thus, it’s not hard to understand that the best, most effective research opportunities exist in the field where Brucellosis is thriving and spreading, just as it is in the Greater Yellowstone Area. The need for brucellosis research was outlined in the National Academy of Sciences’ report “*Revisiting Brucellosis in the Greater*

*Yellowstone Area,”* which concluded that “brucellosis research is not only critical but also should be expanded in response to the spread of brucellosis in the Greater Yellowstone Area.”

### **Limitations and restrictions...**

An August 18 memo from the Department of Health & Human Services and the USDA (described in the body of this article) clarified that it is not permissible to remove an animal which is naturally infected with a select agent (in this case brucellosis) from its natural environment to an artificially-established environment for the purpose of the intentional exposure or introduction of a select agent to a naive (unexposed) or experimental animal OR to introduce a naive animal to a natural environment where there is an animal that is naturally infected with a select agent for the purpose of the intentional exposure or introduction of a select agent to the naive or experimental animal. ... Let that sink in for moment and consider how this policy clarification from the feds literally guts some of the most effective, cutting edge research opportunities that are either already underway or were planned.

These limitations, according to the U.S. Animal Health Association, leave the Biosafety Level 3 (BSL-3) Ag Research Service facility at Ames, Iowa, as the only U.S. facility capable of conducting brucellosis pathogenesis (manner of development of a disease) studies in a laboratory setting. Further, these restrictions preclude any pathogenesis studies under field conditions based on natural transmission of disease in either wildlife or livestock. Therefore, studying vaccine response in cattle, elk, or domestic bison in the Greater Yellowstone Area due to natural infection is no longer possible. So, as brucellosis continues to expand, the scientific research tools previously available to address the problem have become unavailable.

Everywhere we look these days, bureaucrats in Washington D.C. are making unreasonable and unjustifiable decisions that, in my corner of the world, seem to carelessly and thoughtlessly put an entire industry at risk. At the very best, the decisions being made with regard to Brucellosis research set the stage for a major setback in terms of any progress being made to control and eradicate this scourge of the cattle business.

### **The problem begins...**

In January 2016, the USDA-Animal and Plant Health Inspection Service (APHIS) proposed updates to its select agents registration list and to its select agent program regulations. Among the select agents being reviewed were *Brucella abortus* and *Brucella suis*. As I unwound this story after receiving a tip from a reader, I was reminded of President Reagan when he quipped about how Americans should be terribly afraid when a bureaucrat knocks on the door and says, “I’m from the government, and I’m here to help you.” It’s scary enough when the feds start tinkering with research programs vital to an industry, but when they start dismantling ongoing brucellosis research, it’s downright terrifying.

### **The Federal Select Agent Program...**

The Federal Select Agent Program (FSAP) is a collaborative effort—between the Centers for Disease Control & Prevention (CDC), the Division of Select Agents & Toxins, and APHIS Ag Select Agent Services—to regulate the possession, use, and transfer of biological agents also called select agents and toxins. FSAP administers the select agents and toxins regulations in close coordination with the FBI’s Criminal Justice Information Services Division.

The select agents and toxins regulations identify those biological agents and toxins that the Health & Human Services (HHS) Secretary and the APHIS administrator have determined have the potential to pose a severe threat to human health and safety, animal or plant health, or animal or plant products. Any select agent or toxin that is in its naturally-occurring environment is excluded from the requirements of the regulations, provided that agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.



### **CDC & APHIS policy statement...**

An August 18, 2017, memo—signed by Samuel Edwin, Director, CDC's Division of Select Agents & Toxins, and Freeda Isaac, Director, APHIS-Select Agent Services—outlines a shift in FSAP policy. "It is the policy of FSAP that

1) removal of an animal which is naturally infected with a select agent from its natural environment to an artificially established environment for the purpose of the intentional exposure or introduction of a select agent to a naive or experimental animal, or

2) the introduction of a naive animal to a natural environment where there is an animal which is naturally infected with a select agent for the purpose of the intentional exposure or introduction of a select agent to the naive or experimental animal

does not meet the exclusion criteria... Accordingly, any work with an animal naturally infected with a select agent for the purpose of the intentional exposure or introduction of a select agent to any naive or experimental animal may only be conducted after approval by the CDC or USDA (divisions on select agents and toxins) for an entity or individual registered with either agency for possession and use of such select agent. If an animal is confirmed to be naturally infected with a select agent, there may be additional transfer and/or transport restrictions based upon specific USDA and state requirements."

And with that under-everyone's-radar policy statement, USDA and the CDC went about implementing their "*Implementation Plan to Disband the Wildlife/ Livestock Investigations Teams*" (WiLDIT) working at research facilities in Fort Collins, CO, and Corwin Springs, MT.

### **Disbanding plans...**

The plan, said the bureaucrats, addresses the agencies' decision to immediately ramp down the work of the WiLDIT, close the two research facilities, and disband the Corwin Springs, MT, unit. The document includes plans for the reassignment of the WiLDIT personnel, the closure of current research projects, the disposition of research animals and property, and the handling of other logistics and notifications. Under the plan, personnel will be moved out of the WiLDIT group by the end of FY2017, and the disposition plan for all animals was to be determined by July 31, 2017. "The sero-positive and sero-negative bison at the Montana facilities would be disposed of or transferred per agreements with the National Park Service and the Environmental Assessment. Colorado State University will receive the 60-day termination notice required in the current memo of understanding with USDA and the CDC.

Among the research projects being "wrapped up" are Brucellosis infection and transmission dynamics in elk; development of DryDart technology to deliver brucellosis vaccine to bison; use of assisted reproductive techniques to produce brucellosis-free bison with Yellowstone genetics; evaluation of duration of infertility produced by GonaCon, an immune-contraceptive vaccine, in bison; and evaluation of killed preparations of *Brucella abortus* in mice. With regard to the Surveillance, Preparedness, and Response Services Bison Research Facility at Corwin Springs, MT, the plan dictated that Dr. Don Herriott "will take the lead in closing down this project according to the agreements in place... Samples will be collected as indicated in the protocols and environmental assessment... Based on information we currently have but subject to change in sero-status after the current calving season, there are 33 GonaCon-treated bison that need to be incinerated or sent to landfill and 33 sero-negative non-treated females, 10 sero-negative bulls, and 31 sero-positive non-treated females to handle."

(Author's Note: Sero-positive animals have a positive blood serum reaction for the presence on an antibody, which shows that the animal has been exposed to disease at some point. Sero-negative means the animal being tested does not have the presence of antibodies in its blood. Sero-prevalence is the number of animals in a population that test positive for a specific disease based on blood serum specimens and is often presented as a percent of the total specimens tested or as a proportion of the

population. GonaCon is a method of contraception being studied as a method to control the spread of brucellosis. LZ)

### Against the best advice...

It stands to reason that the National Assembly of State Animal Health Officials ((NASAHO) would be the group with the best advice for a federal agency wanting to tinker with something as important as brucellosis research. NASAHO is an organization composed entirely of the state and territorial animal health officials of the United States. The group's mission is to work collectively to safeguard public and animal health as well as the food supply, and it accomplishes this by working with federal, state, and industry partners to develop science-based policies to address issues that affect public and animal health, public safety, and commerce. It goes without saying that NASAHO is uniquely qualified to assess the impact of animal health threats in individual states and territories, as well as how those threats affect the nation.

**Four weeks** before USDA and the CDC-FSAP administrators handed out their August 18 memo, NASAHO sent a letter to Ag Secretary Sonny Perdue, warning him about the consequences of the new policy. "While brucellosis is an expanding zoonotic disease in the Greater Yellowstone Area, the NASAHO is deeply troubled by USDA's decision to decommission critical brucellosis research taking place in Montana and Colorado. Findings from prior research efforts have directly affected decisions relating to management of brucellosis, including needed separation in time between the use of lands by infected wildlife and subsequent use by cattle, potential for remote vaccination of wildlife, and most recently, the ability of bull bison to transmit brucellosis. One ongoing study examines transmission of brucellosis from bison treated with contraceptive agents. Non-surgical reproductive control via contraception has the potential to affect not only population growth, but also spread of brucellosis within and from a wildlife population.

The timing of USDA's announcement to decommission brucellosis field studies strikingly conflicts with the recent completion of the National Academy of Sciences (NAS) report, *Revisiting Brucellosis in the Greater Yellowstone Area*, **which concluded that brucellosis is spreading in wildlife and that the field needs more research on elk and bison rather than less.** The NAS report, funded by APHIS, 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 Greater Yellowstone Area."

NASAHO wrote further, "Once decommissioned, field research on brucella will NOT be resumed in the foreseeable future because of high start-up costs, loss of invaluable expertise, and competing interest for limited funding. **The decision to decommission brucellosis studies puts states that share the geographical range of elk in the Western United States at increased risk.**"

Also of concern to NASAHO is that the void in brucellosis research created by the decommissioning plan will be vulnerable to entities that have previously demonstrated an interest in expanding the range of wildlife in the name of conservation but have little regard for the impact of disease to domestic livestock. "These entities will have drastically different priorities than to protect agriculture," the group wrote. NASAHO advised Perdue that it was strongly requesting "that USDA-APHIS continue critical field research on brucellosis based on the expanding range of the disease..."

Despite NASAHO's well-reasoned advice, the Trump administration, via Ag Secretary Perdue, is marching forward with the decommissioning plan. According to the Montana State Veterinarian's Office, the plan is already being carried out in Montana and Colorado.

### Conclusion...

It's critically important we recognize that the spread of brucellosis in wildlife is a significant risk for the entire Western U.S. and to the U.S. cow herd. Without pathogenesis studies under field conditions based on natural transmission of disease in both wildlife and livestock, studying vaccine response in cattle, elk,

or domestic bison in the Greater Yellowstone Area due to natural infection will no longer be possible. It is an incalculable loss with incredible risks.





# MVDL

## MONTANA VETERINARY DIAGNOSTIC LABORATORY

PO Box 997 Bozeman, MT 59711  
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 #: 8-404-14

Owner: USDA, APHIS, VS

Species: WILD - BISON

Breed: BISON

Name/No. 4G10

Age: NEWB(Sex:

Date Sent: 06/16/2014

Date Received: 06/04/2014

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

### Final Report

Case Coordinator: AWL

### CASE SUMMARY

REASON FOR SUBMISSION: Abortion; Brucella abortus seropositive

#### LABORATORY DIAGNOSIS:

Bison abortion; etiology Streptococcus uberis

COMMENT: Heavy pure growth of Streptococcus uberis was isolated from the abomasal contents. Brucella and Campylobacter cultures were negative. The animal is classified as a Brucella reactor based on serologic results. See attached for results.

A. W. Layton, DVM, DACVP\rb

Date In: 06/04/2014

### PATHOLOGY

Date Out: 06/16/2014

Released by: AWL

GROSS: The carcass is of a female, 60 cm crown/rump length, fully haired bison fetus in poor to fair post mortem and good nutritional state. Abomasum contains thick pink fluid. Lungs are collapsed.

HISTOPATHOLOGY: Tissue sections of kidney, adrenal gland, ileum, abomasum, spleen, lung, heart, liver, spinal cord, diaphragm and thymus are examined. Alveolar spaces are collapsed and many spaces contain sloughed epithelial cells, few macrophages, occasional neutrophils, squamous epithelial cells and meconium. Endothelium of veins within the liver have variable degrees of mineralization.

#### MORPHOLOGIC DIAGNOSIS:

Pneumonia, mild with atelectasis

Meconium and squamous inhalation

Autolysis, moderate

Date In: 06/05/2014

### BACTERIOLOGY

Date Out: 06/13/2014

Released by: jl

#### CULTURES

ID/Site	Specimen	Culture Type	Isolate	Growth	Antimicrobial Profile
	abomasal contents	Campylobacter	Negative for Campylobacter sp.		NA
	abomasal contents	Aerobic	Streptococcus uberis	4+ P	NA
	abomasal contents	Brucella	Negative for Brucella sp.		NA

1+ to 4+ = rare colony to confluent growth

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

Date In: 06/04/14

### SEROLOGY

Date Out: 06/13/14

Released by: AF

000921

MVDL Accession #:  
8-404-14

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA, APHIS, VS

## SEROLOGY

### Test Summary

Testname	# of tests	# Negative	# Positive	# Suspect	# A_C	# Undetermined	# Insufficient	Tech
B. ABORTUS CF	1	0	1	0	0	0	0	AF
B. ABORTUS FP	1	0	1	0	0	0	0	DK
B. ABORTUS BAPA	1	0	1	0	0	0	0	DK
B. ABORTUS CARD	1	0	1	0	0	0	0	DK
B. ABORTUS RIVANOL	1	0	1	0	0	0	0	DK

### List of Significant results

Animal Id	Testname	Result	Titer
GR10	B. ABORTUS CF	POS	4+160
GR10	B. ABORTUS RIVANOL	POS	+200
GR10	B. ABORTUS CARD	POS	
GR10	B. ABORTUS BAPA	POS	
GR10	B. ABORTUS FP	POS	159.7

### Final Classification

Animal Id	Classification	Comment
GR10	REACTOR	Classified by Dr. Houle (DBE)





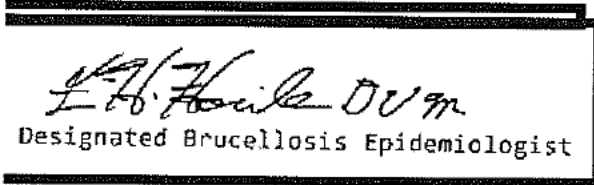
## Knopp, Doug

---

**From:** Frank Houle <(b) (6)@gmail.com>  
**Sent:** Wednesday, June 11, 2014 11:30 AM  
**To:** Knopp, Doug  
**Subject:** Re: Classification of Bison 8-404-14

On 6/11/2014 9:34 AM, Knopp, Doug wrote:

Hi Dr. Houle,  
Here is another bison to classify. It should be pretty



easy.

Doug

Good Morning Doug: Case#8-404-14 Dr. Ryan Clarke Adult Female Bison  
Tube#1 Gr. 10 Is classified as a reactor



This email is free from viruses and malware because avast! Antivirus protection is active.

**MVDL Accession #:**  
8-404-14

**Submitter:**  
PATRICK RYAN CLARKE D.V.M.

**Owner:**  
USDA, APHIS, VS

## Fees

<b>Bacteriology Fee</b>	<b>\$ 0.00</b>
<b>Pathology/Histology Fee</b>	<b>\$ 70.00</b>
<b>Serology Fee</b>	<b>\$ 9.50</b>
<b>Accession Total Fee</b>	<b>\$ 79.50</b>

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

Species: Bison  
County: Park

# MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771  
Phone (406) 994 - 4885 Fax (406) 994 - 6344  
Email: livdiagnosticlab@mt.gov

Collection Date: 7/11/13  
Page 1 of 2

## SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA-APHIS-VS - Gona Con  
ADDRESS:  
CITY/STATE/ZIP: Carwin Springs, MT  
REASON FOR TEST - MANDATORY INFORMATION (See Key)  
BQFS - Gona Con

SUBMITTER'S SIGNATURE: [Signature]  
SUBMITTER'S NAME (PRINT): P. Ryan Clarke  
ADDRESS: (b) (6)  
CITY/STATE/ZIP:  
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL  
NUMBER OR EMAIL ADDRESS: J. Ryan, R. Clarke, B. Frey

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BD	APA	EMD	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	BAPA	Brd	Brd	Brd	Brd					
1	Red 29	Ad	Fe	Bison		Pos	Pos	ISO	3+80	54.5	15.6				
2	Red 28					Pos	Pos	4200	N	Pos	200.4				
3	Green 04					N	N	N	N	N	3.6				
4	Red 02					Pos	Pos	4200	2+10	Pos	172.7				
5	Red 23					Pos	Pos	N	N	Pos	68.5				
6	Red 14					Pos	Pos	4200	4+80	Pos	154.4				
7	Green 06					Pos	N	N	2+10	Pos	30.5				
8	Red 04					N	N	N	N	N	4.5				
9	Red 11					N	N	N	N	N	4.0				
10	Red 27					N	N	N	N	N	6.0				
Laboratory Comments:						Samples	14	14	14	14	14				
						Seropositive	8	6	4	3	8				
						Suspect			1	2	1				
						Seronegative	6	8	9	9	5				
						Undetermined									
						Tested By	<u>DK</u>								

BAPA, FPA, Card, CF, RIV  
please

FEE: \_\_\_\_\_ DATE RECEIVED: 7-11-13 CASE # 8-27

The MVDL is an accredited AAVLD laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

000926



Species: Bison  
County: Park

# MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771

Phone (406) 994 - 4885 Fax (406) 994 - 6344

Email: livdiagnosticlab@mt.gov

Collection Date: 7/11/13  
Page 2 of 2

## SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only (SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA-APHIS-VS-Gona Con  
ADDRESS:  
CITY/STATE/ZIP: CORWIN SPRINGS, MT  
REASON FOR TEST - MANDATORY INFORMATION (See Key)  
BQFS-Gona Con

SUBMITTER'S SIGNATURE: [Signature]  
SUBMITTER'S NAME (PRINT): R. Ryan Clarke  
ADDRESS:  
CITY/STATE/ZIP: (b) (6)  
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL  
NUMBER OR EMAIL ADDRESS: J. Phyllis, R. Clarke, B. Fray

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	EH	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.										
						BAPA	BrV	BrV	BrV	BrV					
							Card	RIV	CF	FP					
11	Red 01	Ad	Fe	Bison		N	N	N	N	N	9.6				
12	Red 31	↓	↓	↓		N	N	N	N	Pos	46.5				
13	Red 05	↓	↓	↓		Pos	N	N	240	Pos	64.5				
14	Red 19	↓	↓	↓		Pos	Pos	F200	N	Pos	157.8				
Laboratory Comments:						Samples									
						Seropositive									
						Suspect									
						Seronegative									
						Undetermined									
						Tested By									

Laboratory Comments:

BAPA, Card, FPA, CF, RIV

FEE: \_\_\_\_\_ DATE RECEIVED: \_\_\_\_\_ CASE # 8-27

The MVDL is an accredited AAVLD laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

000927

Species: Bison  
County: Park

MONTANA VETERINARY DIAGNOSTIC LABORATORY

Box 997 - Bozeman, MT 59771

Phone (406) 994 - 4885 Fax (406) 994 - 6344

Email: livdiagnosticlab@mt.gov

Collection Date: 1-14-15  
Page 1 of 1

SEROLOGY REPORT (SV2A) - Complete Light Shaded Areas Only  
(SEE EXAMPLE and KEY on back Page 4)

OWNER: USDA, APHIS, VS  
ADDRESS:  
CITY/STATE/ZIP: Catwin Springs, MT  
REASON FOR TEST - MANDATORY INFORMATION (See Key)  
BQFS - Gonadon - Cattle Health

SUBMITTER'S SIGNATURE: [Signature]  
SUBMITTER'S NAME (PRINT): P. Ryan Clarke  
ADDRESS: (b) (6)  
CITY/STATE/ZIP:  
RESULT REPORTING OPTIONS: PHONE / FAX / EMAIL  
NUMBER OR EMAIL ADDRESS: R. Clarke, B. Fray, J. Rhyam

CIRCLE DISEASE TEST REQUIRED: If needed, indicate specific test and/or dilution. Example on back.						BRU	BT	ANA	END	PTB	IBR	BVD	BLV	LEPTOSPIROSIS 8 - SEROVARS	OTHER
TUBE NO.	ANIMAL IDENTIFICATION	AGE	SEX	BREED	OFFICIAL VAC.	Final	Ben	Ben	Ben	Ben	CF	FTA			
1	R 421	calf		Bison	* Suspect	Final	Ben	Ben	Ben	Ben	CF	FTA			
2	364					Reactor	Pos	Pos	3+	1:160	P (226.4)				
3	3 R22					Reactor	Pos	Pos	3+	1:10	P (212.6)				
4	3 GDB					Reactor	Pos	Pos	3+	1:320	P (189.2)				
5	3 R20					Reactor	Pos	N	1:20	P (49.4)					
See attach document from DBE for final Laboratory Comments: <u>Classification 2/11/30/15</u>						Samples	5	5	5	5	5	(FPA Delta m value)			
Please do FPA, BAPA						Seropositive									
CF, Card,						Suspect									
* Correction: Tube #1 (R421)						Seronegative									
is classified as "SUSPECT" 2/11/30/15						Undetermined									
						Tested By									

FEE: \_\_\_\_\_

DATE RECEIVED: 1-16-15

CASE # 8-277-15

The MVDL is an accredited AAVLD laboratory and a member of the USDA National Animal Health Laboratory Network. Completing and submitting any submission form or any other means of requesting services creates a contractual agreement for services requested and the specimens submitted become the property of the MVDL. In addition, at no additional expense to our clients, specimens submitted to the MVDL may be subjected to additional testing upon the order of the state or federal animal health officials, or whenever a Foreign Animal Disease is suspected, or in support of surveillance for other animal disease. Serology SV-2A (Rev. 11/09)

000928




**Fuentes, Antonio**

---

**From:** Frank Houle (b) (6) 3@gmail.com>  
**Sent:** Friday, January 30, 2015 11:58 AM  
**To:** Fuentes, Antonio; Horak, Sarah; Knopp, Doug; Liska, Eric; Linfield, Tom; Zaluski, Martin; Thompson, Brent; Clarke, Patrick  
**Subject:** GonaCon Bison Study Case#8-277-15

Tubes#s 2 3G4,3 3R22,4 3G08 and 5 R20 are classified as Reactors Tube#1 R47 is classified as a

  
Designated Brucellosis Epidemiologist

suspect

## 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](mailto: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

<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;

### 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 lmi

**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

**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 Chris Gates  
on 4/21/2015 6:08:15PM

### 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

ID	Taxonomy	Sex	Age
3G02	American Bison	Male	2.0 Years

**Owner:** None Provided

**Specimens Received:** Body; Brain Tissue; L Node; Lung Tissue;

### Bacteriology

#### Aerobic & Anaerobic Culture - Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	4	L Node	Pending	Pending

### Virology

#### Rabies FA

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

### Molecular Diagnostics

#### Canine Herpesvirus (CHV) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	Pending	Pending

#### Ovine Herpesvirus 2 (OHV-2 MCF) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	Pending	Pending

End of Report

**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 4/22/2015 12:12:59PM

### 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

ID	Taxonomy	Sex	Age
3G02	American Bison	Male	2.0 Years

**Owner:** None Provided

**Specimens Received:** Body; Brain Tissue; L Node; Lung Tissue;

### Bacteriology

#### Aerobic & Anaerobic Culture - Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	4	L Node	22-Apr-2015	Anaerobe Results Pending Prelim 04/22/2015 Bacillus species Light growth E. coli Light growth Proteus mirabilis Light growth

### Virology

#### Rabies FA

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

### Molecular Diagnostics

#### Canine Herpesvirus (CHV) - PCR

Animal/Source	Specimen	Specimen Type	Result Date	Results
---------------	----------	---------------	-------------	---------

Owner: None Provided

3G02	2	Lung Tissue	Pending	Pending
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**Ovine Herpesvirus 2 (OHV-2 MCF) - PCR**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	Pending	Pending

End of Report

**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 Mike Russell  
 on 4/23/2015 11:45:40AM

### 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

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;

### Bacteriology

#### Aerobic & Anaerobic Culture - Food Animal

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	4	L Node	22-Apr-2015	Anaerobe Results Pending Prelim 04/22/2015 Bacillus species Light growth E. coli Light growth 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	Mixed Culture Moderate growth IDs pending Prelim 4/23/15

### 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	Pending	Pending

**Ovine Herpesvirus 2 (OHV-2 MCF) - PCR**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	2	Lung Tissue	Pending	Pending

End of Report

**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 Kirsten Reed  
on 4/23/2015 3:12:33PM

**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;

**Bacteriology**

**Aerobic & Anaerobic Culture - Food Animal**

Animal/Source	Specimen	Specimen Type	Result Date	Results
3G02	4	L Node	22-Apr-2015	Anaerobe Results Pending Prelim 04/22/2015 Bacillus species Light growth E. coli Light growth 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	Mixed Culture Moderate growth IDs pending Prelim 4/23/15

**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

**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  
on 4/25/2015 12:03:34PM

**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	Anaerobe Results Pending Prelim 04/22/2015 Bacillus species Light growth E. coli Light growth 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	Mixed Culture Moderate growth IDs pending Prelim 4/23/15

**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

**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

**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 Lisa Jackson  
 on 5/14/2015 4:50:42PM

#### 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

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.

Pituitary gland with cerebral retes: The pituitary gland is normal. There is no evidence of vasculitis within this cerebral rete,



Owner: None Provided

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 lmi

### 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

## 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](mailto: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

**Molecular Diagnostics**

**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.

**Necropsy**

**Necropsy Wildlife / Exotics Gross Examination Only**

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

End of Report

**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. Barbara E. Powers  
sent by Mike Russell  
on 4/24/2015 9:28:41AM

**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>
421	American Bison	Female	

**Owner:** None Provided

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

**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

**Necropsy**

**Necropsy Wildlife / Exotics Gross Examination Only**

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	1	Body		Billing Pending

End of Report

**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. Barbara E. Powers  
sent by Chris Gates  
on 4/24/2015 5:45:10PM

#### 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;

#### 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

#### Necropsy

##### Necropsy Wildlife / Exotics Gross Examination Only

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	1	Body		Billing Pending

End of Report

**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  
 on 4/27/2015 4:15:48PM

#### 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

ID	Taxonomy	Sex	Age
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

#### BSL3

##### 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

**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

**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 4/28/2015 10:07:09AM

#### 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

**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		Billing Pending

End of Report

**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 Lisa Jackson  
on 5/12/2015 5:18:50PM

#### 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;

#### 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

**Molecular Diagnostics**

**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.

**Necropsy**

**Necropsy Wildlife / Exotics Gross Examination Only**

Animal/Source	Specimen	Specimen Type	Result Date	Results
421	1	Body		Billing Pending

End of Report

## Laboratory Report Final

*This report supersedes all  
previous reports for this case*

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

**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

**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

**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 5/1/2015 11:31:15AM

### 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

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 No Salmonella Isolated



Owner: None Provided

Piglet Found Dead 4/21/15	4	Mesenteric	27-Apr-2015	Mixed enterics Moderate growth No Salmonella Isolated
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**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	Mesenteric	28-Apr-2015	Clostridium perfringens 2+
4/21/15				

End of Report

Bison Serum to Steve Hennager for Brucella serology  
From Jack Rhyan

Animal ID	Date Collected
4R21	5/28/15
4R16	5/28/15
420	1/27/15
62	1/27/15
69	1/27/15
3R21	1/27/15
66	1/27/15
63	1/27/15
5R18	1/27/15
61	1/27/15
3R13	1/27/15
3R26	1/27/15
65	1/27/15
130	1/27/15
3R30	1/27/15
3R7	1/27/15
3R25	1/27/15
3R24	1/27/15
52R	5/6/15
47	5/6/15
R57	10/9/14
156	10/9/14
4R7	5/27/15
3G02	1/27/15

Bison Tissues to Chris Quance for Brucella culture  
From Jack Rhyan

Animal ID	Tissue Type	Date Collected
4R21	Hep whole blood	5/28/15
4R16	Hep whole blood	5/28/15
420	Hep whole blood	1/27/15
62	Hep whole blood	1/27/15
69	Hep whole blood	1/27/15
3R21	Hep whole blood	1/27/15
66	Hep whole blood	1/27/15
63	Hep whole blood	1/27/15
5R18	Hep whole blood	1/27/15
61	Hep whole blood	1/27/15
3R13	Hep whole blood	1/27/15
3R26	Hep whole blood	1/27/15
65	Hep whole blood	1/27/15
130	Hep whole blood	1/27/15
3R30	Hep whole blood	1/27/15
3R7	Hep whole blood	1/27/15
3R25	Hep whole blood	1/27/15
3R24	Hep whole blood	1/27/15
3G02	Hep whole blood	1/27/15
4R7	Hep whole blood	5/27/15
156	Hep whole blood	10/9/14
157	Hep whole blood	10/9/14

Tissues to Chris Quance for Brucella testing  
From Jack Rhyan

[illegible]

Gonacon Bison Study Montana Project  
Bison Quarantine Facility, Corwin Springs, MT  
May 15, 2012

[illegible]

Blood: 1 green top, 2 red tops

Vaginal Swabs: 1 dacron swab in WHO media

Gonacon Vaccine: 3 ml left hip

Gonacon Bison Study Montana Project  
Bison Quarantine Facility, Corwin Springs, MT  
May 15, 2012

[illegible]

Blood: 1 green top, 2 red tops

Vaginal Swabs: 1 dacron swab in WHO media

Gonacon Vaccine: 3 ml left hip

Gonacon Bison Study Montana Project  
Bison Quarantine Facility, Corwin Springs, MT  
May 15, 2012

Animal ID	Blood	Vax/Control	Vaginal Swab	Comments

Blood: 1 green top, 2 red tops

Vaginal Swabs: 1 dacron swab in WHO media

Gonacon Vaccine: 3 ml left hip

Study Protocol: J Rhyan

Animal usage (please complete the following box):

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
1. Number approved <b>FOR TOTAL PROJECT</b> on current approval notification <b>plus</b> any subsequent amendments	104			
2. Number of animals used during first IACUC approval year	40			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	40			
4. Number of animals used during third approval year	42			

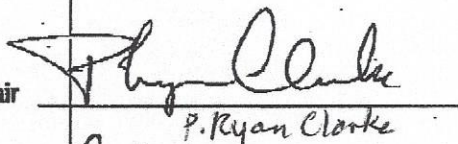
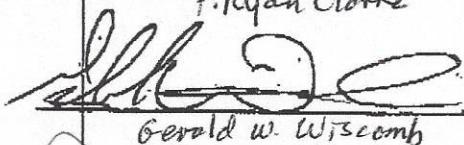
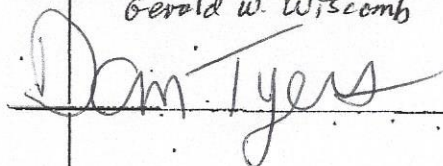
Note: Additional animals (up to 62) will be collected in winter/spring 2014 to replicate the study as described in the original protocol.

Study Director

Date 12/12/13

Concur

IACUC Chair

  
P. Ryan ClarkeDate 12/16/13  
Gerald W. Wiscomb12/17/13  
Dan Tyers1/25/14



**MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS**

**Wildlife Bureau**

P. O. Box 200701 · Helena, MT 59620-0701 · (406) 444-2612

**SCIENTIFIC COLLECTOR'S PERMIT**

**Permit # 2014-022**

**IACUC #** USDA APHIS (5/2014)

**Fee:** No Fee

**Federal Permit #:** NA ()

**Date Issued:** 1/1/2014

**Permitted Activities:** Scientific Collection

**Date Expires:** 12/31/2014

Multiple Year Permit (see note in conditions)

**Permit issued to:** Jack Rhyan

**Address:** USDA APHIS Veterinary Services  
4101 Laporte Ave  
Fort Collins, CO 80521

**Phone number:** 970-266-6140

**Email:** jack.e.rhyan@aphis.usda.gov

**Associated with:** USDA APHIS Veterinary Services

A report of activities conducted under the provisions of this permit must be sent to Montana Fish, Wildlife and Parks, Attn: Wildlife Bureau, POB 200701, Helena, MT 59620-0701 by December 31 annually. The report should list the number of animals handled, including species, date, location (GPS location in UTM coordinates or latitude-longitude if possible; or the legal description in Township, Range, Section and quarter section; otherwise a detailed description of location), other known biological information (sex, age, etc.) and/or cause of death if known. This information will be used for administrative purposes, and to supplement location information housed in the Montana Natural Heritage Program on species.

**Subpermittees:**

Dr. Pauline Nol , John Treanor , Rick Wallin , Chris Geremia , Becky Frey , Doug Blanton , Matt McCollum , Dr. Ryan Clarke

Copies of this permit must be in possession while engaged in activities.

This permit is not transferable.

Montana Fish, Wildlife & Parks

Ken McDonald

Administrator, Wildlife Division



Permit# 2014-022 Page 1 of 3

CC: Howard Burt  
Justin Gude

**MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS**

**Wildlife Bureau**

P. O. Box 200701 · Helena, MT 59620-0701 · (406) 444-2612

**SCIENTIFIC COLLECTOR'S PERMIT**

**Permit # 2014-022**

**Permit Conditions:**

Study period: 2014

Study area: FWP region 3

Authorized Collection: Authorized to collect not more than 108 bison between January 2012 and May 2014 and transport them from Yellowstone National Park to the bison quarantine facility for Gona Con Trial.

Authorized to possess those animals and any subsequent offspring through 2019.

By end of study, seropositive animals shall be euthanized and useable meat donated to food banks. Disposition of seronegative animals including seronegative offspring shall be determined by MFWP.

Permit Expiration Date: This work has been approved for the life of this project. However, an updated permit must be issued annually and a current year permit must be in possession of the applicant to conduct work. To receive updated permits each year prior to the expiration date please notify MFWP in writing (email or hard copy) of your intent to continue work when submitting your annual report. Please also notify MFWP of any changes to subpermittees, methodology, or project objectives. MFWP reserves the right to request a new application and project proposal in the case of significant changes to methodology or project objectives.

Requests to continue work through an updated permit can be made prior to annual report submission for work that is ongoing during the months of December and January.

Copies of this permit must be in possession while engaged in activities.

This permit is not transferable.



Permit# 2014-022 Page 2 of 3

CC: Howard Burt  
Justin Gude

**MONTANA DEPARTMENT OF FISH, WILDLIFE and PARKS**

**Wildlife Bureau**

**P. O. Box 200701 · Helena, MT 59620-0701 · (406) 444-2612**

**SCIENTIFIC COLLECTOR'S PERMIT**

**Permit # 2014-022**

**Other Relevant Montana Code Annotated and Administrative Rules of Montana:**

**MCA 87.2.806- MCA 87.5.109-**

**Copies of this permit must be in possession while engaged in activities.**

**This permit is not transferable.**



**Montana Fish,  
Wildlife & Parks**

**Permit# 2014-022 Page 3 of 3**

**CC: Howard Burt  
Justin Gude**



## MONTANA VETERINARY DIAGNOSTIC LABORATORY

1911 WEST LINCOLN, BOZEMAN, MT 59718  
P.O. Box 997, BOZEMAN, MT 59771  
WEB: [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)



PATRICK RYAN CLARKE D.V.M.  
PO BOX 202001  
HELENA MT 59601

**CASE: 16-19788**

**Name/ID:** Green 09 - with fetus

**Species:** American Bison

**Sex:** Female **Age:** Adult

**County:** Park

**Owner:** USDA, APHIS, VS - R. Clarke

**FINAL REPORT 09/02/16**

**Accessioned:** 06/08/16

**Authorized by:** SS

**Previous Reports**

07/08/16

09/02/16

### CASE SUMMARY

Verified on: 09/02/16 by: SS

#### ADDITIONAL INFORMATION:

Referral culture has been completed on this case, and these results are attached to this final report.

Stephen K. Smith, DVM, Diplomate, ACVP

Verified on: 07/07/16 by: SS

#### REASON FOR SUBMISSION:

Brucella culture

#### LABORATORY DIAGNOSIS:

Pending

#### COMMENT:

Despite the reconfirmation of this cow's serologic status, initial, in-house bacterial cultures fail to isolate Brucella from either of these animals, and these results are attached to this preliminary report. Referral culture is still pending, and these results will be posted when available.

Stephen K. Smith, DVM, Diplomate, ACVP

### PATHOLOGY

Verified on: 07/07/16 by: SS

#### GROSS DESCRIPTION:

This is the carcass of an adult, intact female bison (Green tag #09), with a full term calf that is partially protruding from the vagina. Much of the subcutaneous and deeper connective tissue surrounding the vagina and cervix is hemorrhagic and edematous. No

000986

other significant gross changes are present, and representative samples were taken and submitted for referral Brucella culture.

CLINICAL MICROBIOLOGY

---

Brucella Culture					Verified on: 06/20/16 by: JR
Animal ID	Specimen	Isolate #	Organism	Amount	
Green 09 - with fetus	lung		Negative for Brucella sp.		
Green 09 - with fetus	liver		Negative for Brucella sp.		
Green 09 - with fetus	spleen		Negative for Brucella sp.		
Green 09 - with fetus	caruncle		Negative for Brucella sp.		
Green 09 - with fetus	Abomasal Contents		Negative for Brucella sp.		

SEROLOGY

---

		Carcass	Verified on: 06/09/16 by: AF
Animal	BRUFP		
Green 09 - with fetus	Positive		
Bru. FPA Delta mP value at 89.2 mP. Afs 06/09/2016			

REFERRAL

---

Referral Aerobic Culture		lung	Verified on: 08/30/16 by: JM
Animal ID	Test	Result	
Green 09 - with fetus		Negative-See attached	

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

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

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

The USDA is an equal opportunity provider and employer.

FINAL REPORT

**Laboratory Test Report**

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

**Owner**USDA, APHIS, VS - Ryan Clarke  
, MT**Accession Number:** 16-019153**Animal Location**

Park County MT, US

**Date Collected:** 06/08/2016**Date Received:** 06/10/2016**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**Date Completed:** 08/30/2016**Collected By:** Dr. Stephen K. Smith,**Purpose:** General Diagnostic**Referral Number:** 16-19788

This is not a billable case.

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

Sample: 16-19788,G09 Animal ID: G09 Brucella Case Number: B16-0390 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

**Lymph Node / Lymph Node- S. Mammary**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Internal Iliac**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Internal Iliac**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Retropharyngeal**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Prescapular**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Mandibular**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Popliteal**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Prefemoral**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Mesenteric**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Parotid**

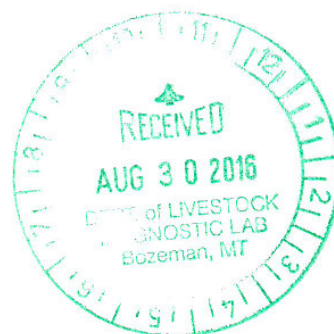
Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Bronchial**

Brucella Isolation Result

No Isolation Made

**Lymph Node / Lymph Node- Hepatic**

16-19788

06/08/16

Page 1 of 2

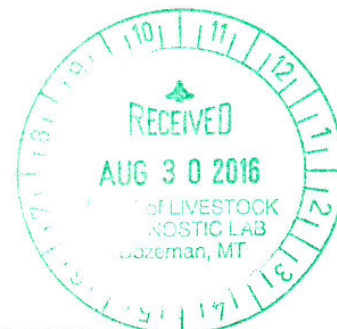
Date Generated: 8/30/2016

000988

Case No: 16-19788 - ,

Page 3 of 5

Brucella Isolation Result	No Isolation Made
<b>Uterus/Vagina / Uterus</b>	
Brucella Isolation Result	No Isolation Made
<b>Tissue / Cervix</b>	
Brucella Isolation Result	No Isolation Made
<b>Ovary/Oviduct / Ovary</b>	
Brucella Isolation Result	No Isolation Made
<b>Placenta / Placenta</b>	
Brucella Isolation Result	No Isolation Made
<b>Tissue / Placental Cotyledon</b>	
Brucella Isolation Result	No Isolation Made
<b>Tissue / Uterus- Caruncle</b>	
Brucella Isolation Result	No Isolation Made
<b>Mammary Gland / Mammary Gland</b>	
Brucella Isolation Result	No Isolation Made
<b>Spleen / Spleen</b>	
Brucella Isolation Result	No Isolation Made
<b>Kidney / Kidney</b>	
Brucella Isolation Result	No Isolation Made
<b>Liver / Liver</b>	
Brucella Isolation Result	No Isolation Made
<b>Swab / Swab- Uterine</b>	
Brucella Isolation Result	No Isolation Made
<b>Tissue / Ileum</b>	
Brucella Isolation Result	No Isolation Made
<b>Swab / Swab- Rectal</b>	
Brucella Isolation Result	No Isolation Made
<b>Feces / Feces</b>	
Brucella Isolation Result	No Isolation Made



Sample: 16-19788,G09 Fetus Animal ID: G09 fetus Brucella Case Number: B16-0391 Specimen Type: Tissue Species: Bison

Brucella Isolation Result No Isolation Made

Individual specimen results are listed below:

**Fetus / Tissue- Not Identified**

Brucella Isolation Result No Isolation Made

**Fluid / Fluid- Abomasal**

Brucella Isolation Result No Isolation Made

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

Help Us Help You

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

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

16-19788  
~~16-19153~~  
8/30/16

FEES:

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B. Abortus FP	1.60
Brucella Culture	80.00
Shipping	32.20
Referral Culture	0.00
Case Summary	0.00
Necropsy LA >500#	157.50
Incineration per pound	234.00
Additional Information	0.00
Total	505.30

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



CERTIFICATE OF VETERINARY INSPECTION

81-454412  
81-35147

TO STATE VETERINARIAN, HELENA, MONTANA

CONSIGNOR NAME AND ADDRESS <u>APHIS, VS, BQFS</u> <u>Corwin Springs,</u>		CONSIGNEE NAME AND ADDRESS <u>APHIS, VS, NWRC</u> <u>4101 La Porte Ave</u>		PERMIT NO.	DATE ISSUED <u>8 Jan 2014</u>
ORIGIN ADDRESS (IF DIFFERENT THAN ABOVE) <u>Montana</u>		DESTINATION ADDRESS (IF DIFFERENT THAN ABOVE) <u>FT Collins, CO 80521</u>		BRAND INSP. NO.	DATE INSPD. <u>8 Jan 2014</u>
PURPOSE OF MOVEMENT: <input type="checkbox"/> BREEDING <input type="checkbox"/> SLAUGHTER <input type="checkbox"/> FEEDING <input checked="" type="checkbox"/> EXHIBITION, ETC. <u>Research</u>		AREA OF ORIGIN STATUS: <input type="checkbox"/> TB MODIFIED ACCREDIT <input type="checkbox"/> TB FREE <input type="checkbox"/> BRUCELLOSIS FREE <input type="checkbox"/> PRV STAGE V <input checked="" type="checkbox"/> OTHER: <u>DSA</u>		CARRIER: <input checked="" type="checkbox"/> TRUCK <input type="checkbox"/> OTHER: NAME & ADDRESS: <u>APHIS, VS</u> <u>4101 La Porte Ave</u> <u>FT Collins, CO 80521</u>	VACCINATION OR TREATMENT FOR (EXCEPT BRUCELLOSIS) PRODUCT: DATE: RECORD NEGATIVE TEST RESULTS LAB:
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: <u>Bison</u>		ORIGIN OF SHIPMENT: A) County: <u>Park</u> B) Market:			

EAR TAG NO. TATTOO OR OTHER PERMANENT IDENTIFICATION	LINE NO.	REGISTRATION NAME AND NUMBER OR DESCRIPTION	VACCINATION TATTOO SYMBOL OR DATE	AGE	SEX	BREED	Disease: Type of Test: DATE	Disease: Type of Test: DATE
840 003 003 334 845	1	Research Bison	N/A	Colt	M	Bison		
840 003 003 334 842	2				M			
840 003 003 334 844	3				M			
840 003 003 334 843	4				M			
840 003 003 334 846	5				Fe			
840 003 003 334 847	6				M			
840 003 003 334 849	7				M			
840 003 003 334 858	8				M			
840 003 003 334 855	9				Fe			
840 003 003 334 848	10				M			
840 003 002 600 802	11			Ad	Fe			
840 003 002 600 804	12			Ad	Fe			
	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: 8 Jan 2014 Accredited Veterinarian Signature: Phy Clarke  
Printed Last Name: CLARKE License #: 1081  
Address: (b) (6) Tel. No.: (b) (6)

OWNER/AGENT STATEMENT (where applicable)

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

Signature of Owner/Agent: [Signature]  
Address: Emigrant, MT  
Date: 8 Jan 2014



## **Wildlife/Livestock Disease Investigations Team (WiLDIT)**

### **Veterinary Services**

#### **National Wildlife Research Center, Fort Collins, CO**

### **Mission**

*Developing science-based solutions to disease problems at the wildlife/livestock interface*

### **Administrative History**

In 1997, following the '96/97 winter when over 1000 bison from Yellowstone National Park died or were sent to slaughter, Dr. Joan Arnoldi, Deputy Administrator of APHIS/VS created the first position of the WiLDIT under the Western Regional Director, Dr. Bob Nervig. The position was located at the NWRC as part of the "One APHIS" concept. The purpose of the position was to help with GYA wildlife issues including continuing research projects begun in 1995. In 1999, when the Regional Director, Dr. Bill Buish, went to NVSL, Dr. Arnoldi placed the WiLDIT position under the VS Deputy's supervision and expanded the duties to include engagement in wildlife/domestic animal interface and game farm disease issues in which VS was involved. In 2000, Dr. Alfonso Torres replaced Dr. Arnoldi and reorganized his staff. Dr. Torres placed the position under Dr. Mike Gilsdorf of the National C Animal Health Program. In 2001, Dr. Torres approved the WiLDIT to begin work on foot and mouth disease (FMD) in North American wildlife. In 2007, with Dr. Gilsdorf's retirement, National Animal Health Program was reorganized and the WiLDIT was placed under Dr. Jerry Diemer, the Assistant Regional Director of the Western Region and leader of the Greater Yellowstone Area core team. Upon Dr. Diemer's retirement in , WiLDIT was placed under Dr. Brian McCluskey, Regional Director of the Western Region. In Dr. Don Herriot became Assistant Regional Director of the Western Region and oversaw WiLDIT activities until 2013, when VS reorganized. WiLDIT resides currently in the Science, Technology, and Analysis Services group of Veterinary Services and is supervised by Dr. Suelee Robbe-Austerman at the National Veterinary Services Laboratory.

Commented [pn1]: What does the C stand for?

Commented [pn2]: Date?

Commented [pn3]: Date?

### **Staff**

Jack Rhyan, DVM, MS-Wildlife Pathologist  
Matt McCollum, MS-Wildlife Biologist  
Pauline Nol, DVM, MS, PhD-Wildlife Epidemiologist  
Karl Held-Animal Health Technician  
Kyle Kelly-APHIS Pathways Student Intern

### **Activity Areas**

**1. Developmental Work** - Coordinate and/or conduct developmental work to address VS-specific problem areas, i.e. vaccine development for wildlife (brucellosis, TB, FMD), test disease diagnostics and detection methods for wildlife (i.e. volatile organic compounds, oral fluids, infrared imaging technology, molecular methods), strategies to eliminate brucellosis from GYA wildlife (i.e., oral vaccination, immunocontraception). Collaborators include, but are not limited to, APHIS-VS, ARS, APHIS-WS, NPS, USGS, Colorado Division of Parks and Wildlife, Colorado State University, Technion-Israel, IREC-Spain, The Nature Conservancy.

**2. Consultation** – Provide advice and consultation to Agency on interface disease issues; serve as liaisons with WS; serve as liaisons with State and Federal wildlife agencies and NGO's.

**3. Training** – Serve as training resource for agencies and universities concerning interface diseases (i.e. wildlife disease instruction at CSU, *Brucella* epi, and FAD courses).

**4. Workshops/Meetings** – Participate in and present information and research findings to various relevant audiences; develop collaborations with colleagues.

**5. One Health** – Conduct developmental work, trainings, and consultations, assist with One Health Office operations, and participate in meetings that apply directly to One Health. Serve as assistant liaisons for VS One Health Office.

### Justification

**Why in Veterinary Services?** In 1997, when the first position was established, Wildlife Services was not involved in any disease work with the exception of rabies. Since then, WS has developed a disease program including work on avian influenza, tuberculosis, West Nile virus, chronic wasting disease, in addition to rabies. WiLDIT has consistently liaised with WS in the development of the WS work and routinely collaborates with WS personnel in laboratory research and field work. This arrangement works well for both sister agencies. Examples of collaborative field work include: volatile organic compounds for disease detection, vaccine development for bovine TB in wildlife, immunocontraception for disease management, benefit-cost analyses of wildlife/livestock disease management.

**Why in Fort Collins?** WiLDIT's location at the NWRC is beneficial to both VS and WS. Additionally, the Fort Collins location allows frequent collaboration with other VS, ARS, CSU, DOI, and State of Colorado domestic animal and wildlife disease experts. A valuable continuing relationship for the WiLDIT is that with the Animal Population Health Institute at CSU. Through a Memorandum of Understanding the two entities share a wildlife research facility and routinely collaborate on projects. The APHI laboratory is a valuable resource for conducting molecular and microbiological work and APHI provides funding for students to work at the wildlife research facility. In addition, WiLDIT and the Animal Reproduction and Biotechnology

Laboratory at CSU have also put into place an MOU concerning collaborative work with embryo transfer technologies in bison for brucellosis management.

### **Students**

In conjunction with Colorado State University, WiLDIT plays a strong role in the education and training of numerous students by providing employment and volunteer opportunities, internship/externship programs, project work for special studies students and graduate students, as well as participation on graduate committees. In the last ten years, through cooperative agreements and grants with CSU, WiLDIT has been able to employ over 20 students as animal care and laboratory technicians, many of whom have gone on to veterinary school.

Since its inception in 1997, WiLDIT has hosted nearly 50 student interns and externs from throughout the United States as well as from Canada, Europe, and South America, eager to learn about research in wildlife, livestock and human health in both government and academic settings. WiLDIT works with the various other wildlife, livestock, and public health agencies in the area in order to coordinate a diverse and comprehensive experience for veterinary, graduate, and undergraduate students.

### **APHI/APHIS Wildlife Research Facility**

The APHI/APHIS Wildlife Research Facility occupies approximately 6.5 acres and is located on the Colorado State University Foothills Campus adjacent to the National Wildlife Research Center in Fort Collins, Colorado. The facility consists of multiple large paddocks with 8-foot high walls, while the entire perimeter of the site is surrounded by a 10-foot high wire fence with added wire to exclude small predators. This facility contains handling equipment for deer, bison, elk, bighorn sheep, feral swine, and other ungulate species. Security is provided by USDA/APHIS, National Wildlife Research Center and CSU security personnel. The animals and facility are maintained by a full time animal care staff and an attending veterinarian. The majority of projects conducted at the facility are approved by the Colorado State University Animal Care and Use Committee. Additionally, some ACUCs are approved by NWRC's IACUC and Quality Assurance staff and some are approved by the Bison Quarantine Facility IACUC in Bozeman, Montana.



United States  
Department of  
Agriculture

Animal and  
Plant Health  
Inspection  
Service

Wildlife Services

National Wildlife  
Research Center

4101 La Porte Ave.  
Ft. Collins, CO  
80521

Ph: 970 266-6000  
Fax: 970 266-6032

Date: November 4, 2011

To: Lowell Miller  
Study Director

Subj: **NWRC IACUC Approval of Study Protocol QA-1923**  
*"Evaluation of GonaCon<sup>TM</sup>, and immunocontraceptive vaccine, in free-ranging bison: A pilot study."*

The NWRC Institutional Animal Care and Use Committee (IACUC) has reviewed this protocol, and all questions, comments, or concerns received by the IACUC members concerning animal care and use issues or procedures have been adequately addressed. As the Chairperson of the NWRC IACUC, I hereby approve this protocol. However, the protocol must still be formally approved by the NWRC Director's Office before any use of animals can begin.

In accordance with the Animal Welfare Regulations, any proposed significant changes to this protocol which involve the care or use of animals need to be described in an amendment and sent to the IACUC Chairperson for review and approval **prior** to those changes being implemented into the study. IACUC non-compliance will be addressed per the procedures as defined in NWRC Policy Memorandum RM 001.

In addition, if a study animal is observed to be experiencing more than slight or momentary pain or distress which was not anticipated or addressed in the original protocol, then the study must stop and the NWRC IACUC Chairperson and/or Attending Veterinarian be contacted immediately. Other significant events such as a high mortality during capture, transport, handling, or while in holding also warrants timely notification to the IACUC Chairperson and/or Attending Veterinarian.

Continuing activities involving the care and use of animals must be re-reviewed on an annual basis. Therefore, each year from the date of this approval you will receive a notice to query the status of this study and provide an assurance of compliance. Long term studies which continue to use animals for more than three years will be reviewed in its entirety for re-approval by the IACUC every third year.

If you have any questions or concerns, please contact me at (970) 266-6169 or [steven.j.greiner@aphis.usda.gov](mailto:steven.j.greiner@aphis.usda.gov). Thank you.

Steve Greiner  
Chairperson, NWRC IACUC



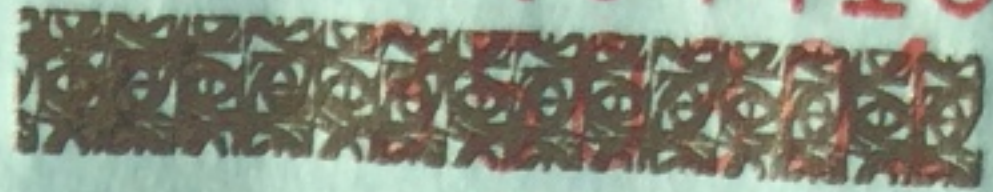
Safeguarding American Agriculture  
APHIS is an agency of USDA's Marketing and Regulatory Programs  
An Equal Opportunity Provider and Employer

Federal Relay Service  
(Voice/TTY/ASCII/Spanish)  
1-800-877-8339



# RY INSPECTION

81 - 454416



TO ACCOMPANY SHIPMENT

MS	PERMIT NO. IP009T6 G	DATE ISSUED 15 Jan 15
CO	BRAND INSP. NO.	DATE INSPD. 15 Jan 15
REPLICA CERTIFICATE YES <input type="checkbox"/> NO <input checked="" type="checkbox"/>		NO OF ANIMALS IN SHIPMENT 10

OTHER: \_\_\_\_\_  
 SS: USDA, APHIS  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

VACCINATION OR TREATMENT FOR  
(EXCEPT BRUCELLOSIS)

PRODUCT: \_\_\_\_\_

DATE: \_\_\_\_\_

RECORD NEGATIVE TEST RESULTS

LAB: \_\_\_\_\_

	AGE	SEX	BREED	Disease:	Disease:
				Type of Test:	Type of Test:
				DATE	DATE
1A	3y	F	Bison		
	3y	F			
	3y	F			
	3y	F			
	3y	F			



This permit identifies restricted animals moved for quarantine/slaughter purposes. The information is needed to identify disease infected/exposed animals that are moved to specific locations in order to control and prevent spread of the disease (9 CFR 71 through 85).

See reverse side for additional information.

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

PERMIT FOR MOVEMENT OF RESTRICTED ANIMALS

USE A SEPARATE FORM FOR EACH SPECIES

1. NAME AND ADDRESS OF SHIPPER OR CONSIGNOR (Include Zip Code)

USDA, APHIS, VS  
Gona Con Research Facility  
Corwin Springs, MT

2. CONSIGNEE (Destination Name and Address, include Zip Code)

USDA, APHIS Research Pens  
4101 La Porte Ave  
Fort Collins, CO

3. MOVED FROM (Name and Location of Premise if other than item 1 above)

4. NAME AND ADDRESS OF OWNER AT TIME CONDITION DIAGNOSED

as # 1

VALID ONLY FOR ABOVE DESTINATION

FORM APPROVED  
OMB NO. 0579-0051

No. E 111145

5. STATE WHERE ISSUED

Montana

6. MOVEMENT TO BE

☒ INTERSTATE ☐ INTRASTATE

7. MOVEMENT FOR

☒ QUARANTINE ☐ SLAUGHTER

8. DISEASE

Brucellosis

9. STATUS OF ANIMALS

No. Reactor No. Exposed No. Other (Specify)

10

10. STATUS OF HERD OF ORIGIN

Research-Infected

11. STATUS OF AREA OF ORIGIN

DSA

12. NO. ANIMALS IN THIS SHIPMENT

10

13. SPECIES (One only)

Bison

14. TRANSPORTATION VEHICLE LICENSE NO. OR OTHER IDENTIFICATION NO.

A362636

15. SEAL NO.

Federal  
Escort

16. VEHICLE REQUIRED TO BE CLEANED AND DISINFECTED AT DESTINATION

☐ YES ☒ NO

(If Yes, Items 32, 33, and 34 are Applicable)

17. ANIMALS TO BE MOVED

COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)	COMPLETE EAR TAG NO.	BREED	SEX	DISEASE BRAND	OTHER IDENTIFICATION (Complete No.)
840-003-003 -405-1000	Bison	Fe	N/A	Red 52	11-958	Bison	F	N/A	4R21
11-607		F		Green 27	11-952		F		4R22
11-608		F		Red 421					
11-609		F		Green 26					
11-610		F		Green 23					
840-003-003 344-951		M		4R03					
11-956		M		4R07					
11-957		F		4R16					

I certify that I have inspected the animals described on this permit and find them eligible to move in accordance with the requirements of State and Federal regulations.

18. SIGNATURE OF INSPECTOR

Thy Clark

19. DATE ISSUED

15 Jan 2015

20. TIME ISSUED

9 AM

VOID AFTER

21. DATE

17 Jan 15

22. TIME

9 AM

WARNING TO OWNER, SHIPPER AND TRUCKER - LIVESTOCK MUST BE DELIVERED TO CONSIGNEE WITHOUT DIVERSION

I understand that it is a violation of Federal law to move the animals identified herein interstate except in accordance with the provisions of applicable Federal Regulations. I also understand that such animals must comply with existing state laws and regulations governing movement of livestock and poultry. I have arranged or will arrange for a copy of this permit to accompany the interstate shipment and be delivered with the above described animals.

23. SIGNATURE OF OWNER OF SHIPPER

Thy Clark

24. TITLE

☐ OWNER ☒ SHIPPER

25. DATE SIGNED

Jan 15, 15

I certify that the animals described on this permit were received and slaughtered/quarantined in accordance with the requirements of the State and Federal regulations on the date indicated in item 29.

26. PLACE ANIMALS RECEIVED

Fort Collins Research Pens

27. DATE ANIMALS ARRIVED

15 Jan 15

28. NO. ANIMALS RECEIVED

10

29. DATE SLAUGHTERED/QUARANTINED

30. DATE AND TIME SEALS BROKE

31. AUTHORIZED SIGNATURE

Thy Clark

32. DATE CLEANED AND DISINFECTED (if required)

33. SIGNATURE OF INSPECTOR

34. DATE SIGNED

15 Jan 15



**Proposed Project:**

**DRAFT**

**Title:** Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

**Investigators:**

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

**Background:**

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

**Major Objectives:**

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

**Minor Objectives:**



1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

#### **Research Plan:**

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon<sup>TM</sup> vaccine (containing 3000µg) and all other bison will remain unvaccinated:

**Pasture A** will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

**Pasture B** will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three

abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for B. abortus after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements and published in the UM&R will be used for bison conservation. (The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.) Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

**Time line:**

**Winter/spring 2011** – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

**Spring 2012** – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

**Winter/Spring 2013-2015** – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December til July each year.

**Summer 2015** – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

**Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

**Proposed Project:**

**DRAFT**

**Title:** Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

**Investigators:**

USDA, APHIS, VS: Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

**Background:**

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

**Major Objectives:**

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

**Minor Objectives:**

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

#### **Research Plan:**

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon<sup>TM</sup> vaccine (containing 3000µg) and all other bison will remain unvaccinated:

**Pasture A** will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

**Pasture B** will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December until July. During the three

abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

**Time line:**

**Winter/spring 2011** – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

**Spring 2012** – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

**Winter/Spring 2013-2015** – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

**Summer 2015** – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

**Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.

2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

**Proposed Project:**

**DRAFT**

**Title:** Evaluation of sterilization by use of and GonaCon™, an immunocontraceptive vaccine, and ovariectomy as means of decreasing the potential for transmission of *Brucella abortus* in bison.

**Investigators:**

**USDA, APHIS, VS:** Jack Rhyan (Principle Investigator), Rebecca Frey, Pauline Nol, Matt McCollum, Ryan Clarke, Luke Wagner

**USDA, APHIS, WS:** Lowell Miller, Kathy Fagerstone

**USDOI, NPS:** Rick Wallen, Jenny Powers

**Background:**

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk; is primarily dependant on the shedding of bacteria following pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary to occasionally long term or permanent infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg GnRH compound. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing shedding and transmission of *B. abortus* which leads to persistence of the disease in populations.

**Major Objectives:**

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.
2. Evaluate the effect of sterilization produced by ovariectomy of *B. abortus*-seropositive female bison on *B. abortus* shedding and transmission in a bison herd.



3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous or sterilization by ovariectomy has on *B. abortus* colonization in naturally-infected female bison; determine if pregnancies following infertility would result in non-infectious parturition.
4. Determine the effect of immune system stimulation via vaccination with Adjuvac (Gonacon's adjuvant) on brucella titers and shedding

#### **Research Plan:**

A total of at least 80 and not more than 100 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 40 to 60 seronegatives and 40 seropositives) and 8 seronegative bulls captured in late winter/spring 2011 and, if needed 2012 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility up to one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 80 female bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into four pastures, each containing 10 seropositives and 10 to 15 seronegative “sentinels” and 2 bulls. Seropositive bison in the four pastures will be treated as follows:

**Pasture A (GonaCon treatment)** will contain 10 seropositive females vaccinated with GonaCon™ vaccine (containing 3000µg), 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

**Pasture B (Untreated control)** will contain 10 seropositive female non-vaccinates, 10 to 15 seronegative female non-vaccinates (sentinels) and 2 seronegative bulls.

**Pasture C (Ovariectomized “gold standard control”)** will contain 10 seropositive ovariectomized female bison, 10 to 15 seronegative female bison and 2 seronegative bulls.

**Pasture D (Adjuvant-only treated controls)** will contain 10 seropositive female bison treated with Adjuvac <sup>™</sup>, 10 to 15 seronegative female bison and 2 seronegative bulls.

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013 – 2017). Bulls will be separated from the cows after breeding season, from December until July. During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. Parturition products at all birth sites will be collected for culture. All bison will be tested by serology and culture in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be cultured immediately at NVSL or maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture pending select agent requirements.

**Time line:**

**Winter/spring 2011** – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate.

**Spring 2012** – Apply treatments: 1. GnRH, 2. Adjuvac, 3. ovariectomize . Place groups in pastures for study; in July, introduce bulls.

**Winter/Spring 2013-2017** – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December through mid-July each year.

**Conclusion of study** – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

**Expected outcomes:**

1. Determine the effectiveness of the permanent sterility produced by ovariectomy and temporary sterility produced by use of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds
2. Determine the effect of prolonged anestrus produced by GonaCon™ and ovariectomy on the survival of *B. abortus* in infected bison. Determine if contracepted female bison become shedders of *B. abortus* after resumption of reproduction.
3. Determine the effect of adjuvant alone on shedding and transmission in seropositive dams
4. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

**Appendix: Sample size calculation:**

Pasture A Seroconversion	Sample size per group				
0.5	407	103	45	24	14
0.4	107	49	28	17	11
0.3	49	29	19	13	9
0.2	28	19	13	10	8
0.1	17	13	10	8	6
0.01	11	9	8	6	5
	0.6	0.7	0.8	0.9	0.99
	Pasture B Seroconversion				

It is anticipated in a fenced enclosure that a single abortion or shedding event will result in the infection of a majority of the sentinels, and no abortion or shedding event will result in no infection of the sentinels. Therefore a sentinel sample size of 10 to 15 should be adequate. Based on previous studies, at least 30% of young seropositive females are expected to abort in the course of 5 years.

**Proposed Project:**

**Title:** Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

**Investigators:**

USDA, APHIS, VS: Jack Rhyan, Pauline Nol, Matt McCollum, Ryan Clarke, Rebecca Frey, Luke Wagner

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

**Background:**

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Transmission of disease in cattle, bison and elk; therefore it is primarily dependant on the occurrence of pregnancy and abortion or calving of infected animals

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in bison. In limited studies, infertility has lasted 3 years or longer following a single injection. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing parturition and thereby preventing transmission of *B. abortus*.

**Major Objectives:**

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

**Minor Objectives:**

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites

2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison
3. Evaluate *B. abortus* transmission to bison bulls during rut.

#### Research Plan:

This general research plan will be followed. A total of 40 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately half seronegative and half seropositive) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and bi-annually thereafter. Bulls will be maintained separately and monitored by serology. In spring 2012, animals will be relocated into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive GonaCon™ vaccine and all other bison will remain unvaccinated:

**Pasture A** will contain approximately 10 seropositive female vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

**Pasture B** will contain approximately 10 seropositive female non-vaccinates, 10 seronegative female non-vaccinates (sentinels) and 3 seronegative bulls.

Female sentinel bison will be fitted with proximity collars programmed to record proximity to one another and to transmitters on vaginal implants. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). During that period, calving, the occurrence of abortions, and serology in the groups will be monitored. In February each year, animals will be pregnancy tested and pregnant animals fitted with vaginal transmitters. Transmitters will alert investigators to abortion or calving events and record exposure of sentinel animals. Animals will be tested by serology in February and in summer following calving. At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. Offspring from the study will be monitored by serology and culture twice a year throughout the study. Offspring that remain or become positive for *B. abortus* by serology or culture after weaning will be euthanized and necropsied. Offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be translocated to tribal and/or public lands or utilized for conservation of the genetics.

Commented [rkf1]: Three years from when? After injection; or after 3 possible births. In other words...if we were to get animals in 2011, when would they be necropsied? Is there any need to keep them more than 3 years?

Commented [rkf2]: Had to read twice to comprehend.....all animals from original capture only?

**Time line:**

**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.

**Winter/Spring 2013-2015** – monitor herds for calves, abortions, and seroconversions.

**Summer 2015** – Euthanize, necropsy and culture study animals; utilize calves for genetic conservation.

**Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

**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:

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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.

**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:**

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#### **Research Plan:**

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. [Routine procedures \(collecting blood, fitting collars, etc.\) will be done in the facility bison chute.](#) Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. [Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available.](#) The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; [no cattle are present within a mile of the facilities.](#) These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

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[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.

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**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.

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**Proposed Project:**

**Title:** Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison.

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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

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#### Research Plan:

This general research plan will be followed; details will be worked out in further consultation with collaborators and a more extensive protocol developed. 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:

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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.



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**Minor Objectives:**

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## Proposed Project:

## DRAFT

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### Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
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#### **Research Plan:**

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available. The animals will be housed and the study conducted in the double-fenced facilities utilized for the **Bison Quarantine Feasibility Study** located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities. In spring 2012, animals will be sorted into two pastures, each containing half the seropositives and half the seronegatives and 3 bulls. Seropositive bison in one pasture will receive a single injection of GonaCon™ vaccine (containing 3000µg) and all other bison will remain unvaccinated:

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Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three

abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for B. abortus after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. {The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society.} Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. Specimens for culture collected during the study will be maintained frozen at -minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.

#### **Time line:**

**Winter/spring 2011** – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate. Conduct pilot studies on captive bison in Fort Collins, CO to perfect fetus proximity detection technology.

**Spring 2012** – Vaccinate with GnRH. Place groups in pastures for study; in July, introduce bulls.

**Winter/Spring 2013-2015** – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December til July each year.

**Summer 2015** – Euthanize, necropsy and culture seropositive study animals, collect ova and semen for genetic conservation.

When seronegative study adults and offspring meet requirements of quarantine, use for bison conservation.

#### **Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.
5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

# Implementation Plan to Disband the Wildlife/Livestock Disease Investigations Team

## SUMMARY

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This plan addresses the decision by the Office of the Deputy Administrator to immediately ramp down the work of the Wildlife/Livestock Disease Investigations Team (WiLDIT), close their research facilities in Fort Collins, CO, and the pens at Corwin Springs, MT, and disband this unit.

The plan includes the reassignment of the WiLDIT personnel, the closure of current research projects, disposition of research animals, property disposition, other logistics, and notifications. Information on the agreements and Memorandum of Understanding (MOU) in place with other entities regarding the projects and disposition of the research animals is included.

Personnel will be moved out of the WiLDIT group by the end of FY 2017. The disposition plan for all animals will be determined by July 31, 2017. After approval of the implementation plan, discussions with certain project collaborators are needed to determine if they want to acquire the research animals and then APHIS will need to approve the proposed disposition. The seropositive and seronegative bison at the Montana facilities would be disposed of or transferred per agreements with the National Park Service (NPS) and the Environmental Assessment (EA). Colorado State University (CSU) will receive the 60-day termination notice required in the current MOU.

It has been determined that a 1010 package and Congressional notification are not required for this organizational change; however, a Civil Rights Impact Analysis (CRIA) is needed and underway.

## PERSONNEL PLACEMENT

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The four permanent employees currently assigned to the WiLDIT group will retain their grades and be reassigned pending approval from the CRIA, which is currently underway. The term employee is in the second year of a possible four-year position that expires March 6, 2018. There is one Saul T. Wilson student who graduates in May 2018. There are eight part-time students hired through the CSU cooperative agreement for their tuberculosis vaccine project.

- Dr. Jack Rhyan will remain with the VS National Veterinary Services Laboratories (NVSL) and will be assigned to oversee the writing of publications related to the completed research, continue to serve as a pathologist on special projects



submitted to NVSL from the field, and serve on a regular basis as a pathologist for NVSL in Ames, IA. His duty station would remain in Colorado.

- Dr. Pauline Nol, Veterinary Medical Officer, and Matt McCollum, Wildlife Biologist, will be reassigned to VS Center for Epidemiology and Animal Health (CEAH) to provide wildlife expertise to CEAH projects. They are meeting with the CEAH Director to determine the appropriate organizational unit at CEAH for reassignment.
- Samantha Bruce, the Saul T. Wilson student, will transfer to CEAH. Since she graduates in May 2018, it is not expected she will work much after this summer.
- Karl Held, Animal Health Technician, will be assigned to the VS Surveillance Preparedness and Response Services (SPRS) District 6 office in Lakewood, CO, with his duty station as his home.
- Morgan Wehtje is on a term appointment on feral swine annual appropriations expiring March 6, 2018. APHIS Human Resources has confirmed that we are obligated to maintain this term position through the expiration date. She is finishing her PhD in disease ecology with extensive experience in modeling, data analytics, and spatial analysis in the next 2 months. She is meeting with the CEAH Director to determine where her skills would best be used. Wildlife Services (WS) may have at least partial salary funding to allow completion of a feral swine collaborative project and VS Science, Technology, and Analysis Services (STAS) will look at options for funding the salary.

## RESEARCH PROJECTS WRAP-UP

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### COLORADO PROJECTS

#### **Evaluation of duration of infertility produced by GonaCon™, an immuno-contraceptive vaccine, in bison**

**Collaborators:** The Nature Conservancy; WS/NWRC

**Location:** Medano-Zapata Ranch (animals owned by The Nature Conservancy) in southern Colorado

**Status:** Started in 2011 with bison from a brucellosis negative herd and to be completed in November 2017. APHIS does not have ownership of these animals. Contact will be made with the owners of the animals (Nature Conservancy) and NWRC about ending this project early. However, since the animals are only rounded up in November of each year, the tissues from the nine GonaCon™ treated bison needed to complete the study may need to be collected when the animals are available as they are mixed on a large range. Since WS/NWRC is a collaborator, transfer of the study conclusion could be discussed with officials there.

**Agreements:** This study is being conducted under NWRC-approved Protocol QA-1923.

This protocol requires that tissues be taken for histopathology. Jack Rhyan will complete the histopathology examination. NWRC and Jack Rhyan will work jointly to write this project report. GonaCon™ treated bison cannot be used for human consumption based on the EA.

**Brucellosis infection and transmission dynamics in elk**

**Collaborators:** Wyoming Game and Fish, USDA Agricultural Research Service (ARS), CSU

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** The elk were moved to the facility, however, the project was halted in February 2017. All seropositive elk were removed by April 2017. Collaborators were notified in February and March of 2017.

**Agreements:** This project did not have any agreements with collaborators. A CSU-approved Institutional Animal Care and Use Committee (IACUC) protocol was in place (#14-4956A). Collection permits were in place with Wyoming Game and Fish (#33-1040-2017; #33-1041-2017) and Colorado Parks and Wildlife (#17TR2152).

**Development of DryDart technology to deliver brucellosis vaccine to bison**

**Collaborators:** ARS/National Animal Disease Center (NADC)

**Location:** ARS/NADC

**Status:** There is an ongoing study at NADC in Ames evaluating injected pelleted RB51 vaccine in comparison to liquid RB51. The study will conclude with challenge in 2018. WiLDIT personnel are not necessary to complete the study as is in NADC facility.

**Agreements:** NA

**Use of assisted reproductive techniques to produce brucellosis-free bison with Yellowstone genetics**

**Collaborators:** CSU – lead

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** This project depends on bison owned by APHIS that will no longer be available for use by CSU.

**Agreements:** There is a five-year MOU with CSU signed by the Western Region Director in effect until October 2018 that requires a 60-day notice for termination. This notification will be sent on approval of the implementation plan. These bison are also under the agreements applicable to Yellowstone National Park (YNP) bison regarding disposition.

**Evaluation of killed *Mycobacterium bovis* vaccine to protect feral swine from bovine tuberculosis**

**Collaborators:** CSU; University Castilla la Mancha, Spain; Neiker Inc., Spain

**Location:** WiLDIT Wildlife Research Facility and CSU

**Status:** Feral swine piglets of Hawaiian origin have been vaccinated and will enter CSU BSL-3 in August 2017 for *M. bovis* challenge. These animals are from a brucellosis- and tuberculosis (TB)-free herd. On entry into the BSL-3 at CSU, these animals will no longer be the primary responsibility of WiLDIT. Animals will be necropsied November-December 2017. Disposition of pigs is by incineration. Tissues will be collected and cultured at NVSL and histopathology read by Jack Rhyan to complete this study. Manuscript will be

prepared and submitted for publication based on data. This study is being conducted under CSU IACUC Approved Protocol #14-5367A.

**Agreements:** A cooperative agreement with CSU covers this project. A master's student is depending on having these animals available for sampling in the BSL-3 for her oral fluids project described below (Two Agreements: Alfano, Marialexia-Learning Plan; Alfano, Marialexia-Placement Agreement).

#### **Development of volatile organic compounds (VOCs) in wild pigs**

**Collaborators:** Rovira i Virgili University, Spain, IREC, University Castilla la Mancha, Spain, CSU; NWRC

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO, and CSU

**Status:** Project with Spain is nearing conclusion. Intended sample collections from current projects will be taken over by NWRC or will not take place.

**Agreements:** Current cooperative agreement with Rovira i Virgili University will be completed and closed out by August 14, 2017, and manuscript(s) prepared and submitted for publication based on data collected from previous projects.

#### **Development of oral fluid collection studies for feral swine**

**Collaborators:** CSU, VS-STAS; University of Florida (UFL); University of Georgia (UGA)

**Locations:** Savannah River Field Station, Georgia, and CSU.

**Status:** Oral fluids are currently being collected from feral swine in the field via a collection device comprised of a wool ball and attractants (swine apples) deployed by UGA and UFL collaborators. A Colorado School of Public Health master's student will also collect oral fluids from pigs via ropes and swine apples for a project called the "evaluation of killed *Mycobacterium bovis* vaccine to protect feral swine from bovine tuberculosis." The student is depending on these data to complete her student practicum, a requirement for graduation. The animal portion of this project will be completed when the study is terminated at necropsy in November 2017. UFL and Savannah River collaborators will participate in a presentation to be given at the International Wildlife Disease Association Conference in Chiapas, Mexico, in July 2017.

**Agreements:** NA

#### **Development of oral fluid collection studies for elk**

**Collaborators:** NA

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Study will evaluate methods of oral fluids collection in elk. Study will be completed by September 30, 2017. This study is being conducted under CSU IACUC Approved Protocol # 17-7157A.

**Agreements:** NA

#### **Evaluation of killed preparations of *Brucella abortus* in mice**

**Collaborators:** CSU

**Location:** CSU, Fort Collins, CO

**Status:** The third of three studies is in the final stage and will be completed by September 2017. There are no live animals left in this study. CSU is conducting cultures.

**Agreements:** A cooperative agreement was in place for this study. The CSU IACUC protocol was #16-6694A.

### **Bison conservation**

**Collaborators:** VS/SPRS; CSU; City of Fort Collins, CO; Larimer County, CO

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Yellowstone-genetics bison have been used to establish or augment four public herds. Twenty bison are going through the APHIS-approved quarantine protocol in the WiLDIT pens. The youngest animals that need to complete quarantine per agreements were born in 2017. This means the project will end in 2022. We will develop a plan to ensure these animals complete quarantine or reach agreement on transferring ownership of these bison or reach agreement for other disposition.

**Agreements:** These bison are covered under YNP agreements referred to under the Montana Projects section and under the 2015 Intergovernmental Agreement that includes APHIS, the City of Fort Collins, Larimer County and CSU. APHIS' responsibilities in the agreement include providing the "seedstock" bison for the project and disease monitoring for all project animals to ensure the bison remain brucellosis free. The agreement signed by Dr. Shere on behalf of Dr. John Clifford provides that any party may terminate the agreement at any time on no less than 6 months advance written notice to the other parties.

### **Development of safe and effective immobilization protocols for wild swine**

**Collaborators:** WS/NWRC, Colorado Parks and Wildlife; Texas A&M University; Wildlife Pharmaceuticals

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Studies will be completed by September 30, 2017.

**Agreements:** NA

### **Use of DryDarts to deliver immobilizing agents to elk**

**Collaborators:** Wildlife Pharmaceuticals

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Study will be completed by September 30, 2017.

**Agreements:** NA

## **MONTANA PROJECTS**

### **Evaluation of GonaCon™, an immuno-contraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison in the Greater Yellowstone Area**

**Collaborators:** VS/SPRS; WS/NWRC; YNP

**Location:** APHIS quarantine facility in Corwin Springs, MT

**Status:** Don Herriot will be coordinating this project termination in accordance with the applicable agreements.

**Agreements:** There are two main agreements governing disposition of the bison from this project. There have been previous agreements with the NPS allowing the removal of bison from YNP for the project that included animal disposition requirements. The most recent

agreement was an Interagency Agreement (IAA) between APHIS and the NPS signed in February 2013 by Dr. Mark Davidson as the Western Region Director with a period of performance through January 31, 2017. The IAA refers to the EA when describing the consignment of the bison based on brucellosis status. The 2012 EA titled *Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of Brucella abortus in Bison in the Greater Yellowstone Area*, and the resulting Finding of No Significant Impact for the Proposed Study (FONSI) signed by Dr. Don Herriott in May 2012 included animal disposition information. In brief, the agreements provide:

- GonaCon™ treated bison will be disposed of by incineration or landfill burial. Per the conditions of approval from the Environmental Protection Agency for this study, they cannot be consumed by humans. If APHIS wanted to handle this any differently, significant discussions would need to take place.
- Brucellosis seropositive bison:
  - EA states
    - “Seropositive animals from the study that have not received GonaCon™ would be distributed to Montana food banks as is routinely done with other YNP seropositive bison.”
  - FONSI states
    - “Both bison that test seropositive for brucellosis and bison treated with GonaCon™ from the study would not be allowed to be consumed by humans and would be humanely euthanized when the study is complete.” Note this is not what is in the EA or the IAA regarding the seropositive non-treated bison. The EA states that there is no danger of transmission of the infection to humans from consuming cooked meat from *B. Abortus* infected bison and the bacteria typically is not found in muscle tissue with normal cooking temperatures killing any existing bacteria.
  - IAA states
    - “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 generic conservation utilizing embryo transfer techniques.”
    - “All or a subset of offspring that remain or become seropositive for *B. abortus* will be maintained and monitored through their first parturition.”
- Bison that test negative for brucellosis exposure:
  - EA states
    - “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.”
  - IAA states
    - “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.”
  - It is noted, “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.”
- An additional requirement is to provide final reports to the Key Official for the NPS, who is the Superintendent of YNP. In response to questions on the EA, APHIS noted that when the study is complete, the results would be published in a peer-reviewed scientific journal and that the disposition of the animals or genetic materials from the study would be made after consultation with bison experts at YNP and conservation organizations such as the American Bison Society, the International Union for Conservation of Nature, or other applicable organizations. Note: APHIS will need to determine the disposition in light of the current situation in the GYA and consideration of State animal health officials’ assessments.

In addition, the study is being conducted under NWRC-approved Protocol QA-1858, and protocol approval from the Bison Quarantine Facility IACUC.

## COOPERATIVE AGREEMENTS STATUS

WiLDIT had four cooperative agreements in place in 2017.

- Detection of TB, Bruc in swine
  - Fundacio URV in Tarragona, Spain
  - Ends August 14, 2017
  - Funds expended
- Inactivated *Brucella abortus* vaccine in mice
  - Colorado State University
  - Ends June 30, 2017
  - Funds will be expended
- Molecular detection of *Mycobacterium bovis*
  - Colorado State University
  - Ends August 14, 2017
  - Funds will be expended
- TB *M. bovis* vaccine in feral swine
  - Colorado State University
  - Ends July 31, 2017

- Funds remaining will be expended with no cost extension to conclude the study in CSU biocontainment unit

## DISPOSITION OF ANIMALS

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### WiLDIT Wildlife Research Facility, Fort Collins, CO

#### **Elk**

All brucellosis-seropositive elk have previously been euthanized. Many of these animals were either born at the WiLDIT facility or have been housed here for some time; therefore they are well suited to captive research. We will attempt to transfer the remaining 34 brucellosis-seronegative elk to another research facility; possibly NADC, Colorado Parks and Wildlife, CSU, or Wyoming Game and Fish Department. There are no prior agreements associated with the disposition of these animals. Since they are now housed within a CWD endemic area, this may limit the number of locations where they can be transferred.

#### **Bison**

Currently, in Fort Collins, 22 seronegative bison are going through the approved APHIS quarantine protocol and there are 11 seropositive bison. Disposition of animals is governed by the 2013 IAA with YNP and the Intergovernmental Agreement referred to in the bison conservation project. APHIS will need to determine if either WS or CSU could potentially continue the quarantine process to meet the agreements. Quarantine of the seronegative animals would be completed in 2022.

#### **Swine**

Two breeding herds exist: Texas feral swine and Hawaii feral swine. Both herds have 8-10 breeding sows and two boars. Because these animals have been trained to be handled, and the Hawaii feral swine are rather valuable for TB research, we will work with our collaborators to see if there is any interest in transferring these animals. This will be completed by the end of September 2017.

### Surveillance, Preparedness and Response Services (SPRS) Bison Research Facility, Corwin Springs, MT

Dr. Don Herriott will take the lead in closing down this project according to the agreements in place and described under the Montana Projects section. Samples will be collected as indicated in the protocols and Environmental Assessment. Based on information we currently have but subject to change in serostatus after the current calving season, there are 33 GonaCon™ treated bison that need to be incinerated or sent to landfill and 33 seronegative non-treated females, 10 seronegative bulls, and 31 seropositive non-treated females to handle according to the NPS agreements.

## PROPERTY DISPOSITION

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WiLDIT personnel will provide their inventory records to Marjorie Swanson, CEAH Administrator Officer, and MaryAnn Waterbury, NVSL Property Manager, for suitable disposition of equipment and supplies at the Colorado pens and the laboratory space at NWRC. NVSL will send one or two individuals trained in property acquisition and disposition to assist the team in this process. STAS will work with the SPRS District 5 Administrator Officer Tim Solinger on the property disposition in Montana.

## OTHER LOGISTICS

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### **Pens in Montana – coordinated with Dr. Don Herriott**

- Leases are up for renewal in July for two of the three pens, with the other expiring in March 2018. It is expected we would renew the two leases to allow for the summer blood tests and return land to original state if requested as provided for in the leases. The Forest Service and NPS may have interest in leasing two of the sites. One of the leases is funded through a cooperative agreement with the Montana Department of Agriculture.
- Current animals in the leased pens are related to the WiLDIT project. Currently, there are no other animals under quarantine in the pens.

### **Pens in Colorado**

- There is no lease for the WiLDIT pens in CO that are on CSU property. There is a MOU with a 60-day termination notice to be provided. This will be provided as soon as the plan is approved and when it has been determined that the last animals will be removed or the ownership of those animals transferred. CSU may elect to maintain the pens on its property or request that the ground be returned to its original state by Veterinary Services.

## NOTIFICATIONS

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On acceptance of the plan, several notifications will take place.

- VS Deputy Administrator Dr. Jack Shere will notify the State Veterinarians in Wyoming, Montana, and Colorado of the disposition of the WiLDIT projects they have been involved with. The Hawaii State Veterinarian will be notified when it is determined if the feral swine from Hawaii are transferred to a collaborator. An update will be provided on the tuberculosis vaccine trial at that time.
- Dr. Jack Rhyan will notify collaborators.
- Dr. Don Herriott will notify the NPS.
- Dr. Shere will provide any notifications necessary for VS.



# Implementation Plan to Disband the Wildlife/Livestock Disease Investigations Team

## SUMMARY

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This plan addresses the decision by the Office of the Deputy Administrator to immediately ramp down the work of the Wildlife/Livestock Disease Investigations Team (WiLDIT), close their research facilities in Fort Collins, CO, and the pens at Corwin Springs, MT, and disband this unit.

The plan includes the reassignment of the WiLDIT personnel, the closure of current research projects, disposition of research animals, property disposition, other logistics, and notifications. Information on the agreements and Memorandum of Understanding (MOU) in place with other entities regarding the projects and disposition of the research animals is included.

Personnel will be moved out of the WiLDIT group by the end of FY 2017. The disposition plan for all animals will be determined by July 31, 2017. After approval of the implementation plan, discussions with certain project collaborators are needed to determine if they want to acquire the research animals and then APHIS will need to approve the proposed disposition. The seropositive and seronegative bison at the Montana facilities would be disposed of or transferred per agreements with the National Park Service (NPS) and the Environmental Assessment (EA). Colorado State University (CSU) will receive the 60-day termination notice required in the current MOU.

It has been determined that a 1010 package and Congressional notification are not required for this organizational change; however, a Civil Rights Impact Analysis (CRIA) is needed and underway.

## RESEARCH PROJECTS WRAP-UP

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### COLORADO PROJECTS

#### **Evaluation of duration of infertility produced by GonaCon™, an immuno-contraceptive vaccine, in bison**

**Collaborators:** The Nature Conservancy; WS/NWRC

**Location:** Medano-Zapata Ranch (animals owned by The Nature Conservancy) in southern Colorado

**Status:** Started in 2011 with bison from a brucellosis negative herd and to be completed in November 2017. APHIS does not have ownership of these animals. Contact will be made with the owners of the animals (Nature Conservancy) and NWRC about ending this project

early. However, since the animals are only rounded up in November of each year, the tissues from the nine GonaCon™ treated bison needed to complete the study may need to be collected when the animals are available as they are mixed on a large range. Since WS/NWRC is a collaborator, transfer of the study conclusion could be discussed with officials there.

**Agreements:** This study is being conducted under NWRC-approved Protocol QA-1923. This protocol requires that tissues be taken for histopathology. Jack Rhyan will complete the histopathology examination. NWRC and Jack Rhyan will work jointly to write this project report. GonaCon™ treated bison cannot be used for human consumption based on the EA.

### **Use of assisted reproductive techniques to produce brucellosis-free bison with Yellowstone genetics**

**Collaborators:** CSU – lead

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** This project depends on bison owned by APHIS that will no longer be available for use by CSU.

**Agreements:** There is a five-year MOU with CSU signed by the Western Region Director in effect until October 2018 that requires a 60-day notice for termination. This notification will be sent on approval of the implementation plan. These bison are also under the agreements applicable to Yellowstone National Park (YNP) bison regarding disposition.

### **Bison conservation**

**Collaborators:** VS/SPRS; CSU; City of Fort Collins, CO; Larimer County, CO

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Yellowstone-genetics bison have been used to establish or augment four public herds. Twenty bison are going through the APHIS-approved quarantine protocol in the WiLDIT pens. The youngest animals that need to complete quarantine per agreements were born in 2017. This means the project will end in 2022. We will develop a plan to ensure these animals complete quarantine or reach agreement on transferring ownership of these bison or reach agreement for other disposition.

**Agreements:** These bison are covered under YNP agreements referred to under the Montana Projects section and under the 2015 Intergovernmental Agreement that includes APHIS, the City of Fort Collins, Larimer County and CSU. APHIS' responsibilities in the agreement include providing the "seedstock" bison for the project and disease monitoring for all project animals to ensure the bison remain brucellosis free. The agreement signed by Dr. Shere on behalf of Dr. John Clifford provides that any party may terminate the agreement at any time on no less than 6 months advance written notice to the other parties.

## MONTANA PROJECTS

### **Evaluation of GonaCon™, an immuno-contraceptive vaccine, as a means of decreasing transmission of *Brucella abortus* in bison in the Greater Yellowstone Area**

**Collaborators:** VS/SPRS; WS/NWRC; YNP

**Location:** APHIS quarantine facility in Corwin Springs, MT

**Status:** Don Herriot will be coordinating this project termination in accordance with the applicable agreements.

**Agreements:** There are two main agreements governing disposition of the bison from this project. There have been previous agreements with the NPS allowing the removal of bison from YNP for the project that included animal disposition requirements. The most recent agreement was an Interagency Agreement (IAA) between APHIS and the NPS signed in February 2013 by Dr. Mark Davidson as the Western Region Director with a period of performance through January 31, 2017. The IAA refers to the EA when describing the consignment of the bison based on brucellosis status. The 2012 EA titled *Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of Brucella abortus in Bison in the Greater Yellowstone Area*, and the resulting Finding of No Significant Impact for the Proposed Study (FONSI) signed by Dr. Don Herriott in May 2012 included animal disposition information. In brief, the agreements provide:

- GonaCon™ treated bison will be disposed of by incineration or landfill burial. Per the conditions of approval from the Environmental Protection Agency for this study, they cannot be consumed by humans. If APHIS wanted to handle this any differently, significant discussions would need to take place.
- Brucellosis seropositive bison:
  - EA states
    - “Seropositive animals from the study that have not received GonaCon™ would be distributed to Montana food banks as is routinely done with other YNP seropositive bison.”
  - FONSI states
    - “Both bison that test seropositive for brucellosis and bison treated with GonaCon™ from the study would not be allowed to be consumed by humans and would be humanely euthanized when the study is complete.” Note this is not what is in the EA or the IAA regarding the seropositive non-treated bison. The EA states that there is no danger of transmission of the infection to humans from consuming cooked meat from *B. Abortus* infected bison and the bacteria typically is not found in muscle tissue with normal cooking temperatures killing any existing bacteria.
  - IAA states
    - “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 generic conservation utilizing embryo transfer techniques.”
- “All or a subset of offspring that remain or become seropositive for *B. abortus* will be maintained and monitored through their first parturition.”
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      - “Consigned to a quarantine location for further diagnostics;
      - Consigned to a managed for public trust conservation program to supplement population genetic diversity;
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      - Utilized in an embryo transfer program for bison genetics conservation.”
      - It is noted, “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.”
  - An additional requirement is to provide final reports to the Key Official for the NPS, who is the Superintendent of YNP. In response to questions on the EA, APHIS noted that when the study is complete, the results would be published in a peer-reviewed scientific journal and that the disposition of the animals or genetic materials from the study would be made after consultation with bison experts at YNP and conservation organizations such as the American Bison Society, the International Union for Conservation of Nature, or other applicable organizations. Note: APHIS will need to determine the disposition in light of the current situation in the GYA and consideration of State animal health officials’ assessments.

In addition, the study is being conducted under NWRC-approved Protocol QA-1858, and protocol approval from the Bison Quarantine Facility IACUC.

## DISPOSITION OF ANIMALS

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WiLDIT Wildlife Research Facility, Fort Collins, CO

## **Bison**

Currently, in Fort Collins, 22 seronegative bison are going through the approved APHIS quarantine protocol and there are 11 seropositive bison. Disposition of animals is governed by the 2013 IAA with YNP and the Intergovernmental Agreement referred to in the bison conservation project. APHIS will need to determine if either WS or CSU could potentially continue the quarantine process to meet the agreements. Quarantine of the seronegative animals would be completed in 2022.

## **Surveillance, Preparedness and Response Services (SPRS) Bison Research Facility, Corwin Springs, MT**

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## **PROPERTY DISPOSITION**

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## **OTHER LOGISTICS**

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### **Pens in Montana – coordinated with Dr. Don Herriott**

- Leases are up for renewal in July for two of the three pens, with the other expiring in March 2018. It is expected we would renew the two leases to allow for the summer blood tests and return land to original state if requested as provided for in the leases. The Forest Service and NPS may have interest in leasing two of the sites. One of the leases is funded through a cooperative agreement with the Montana Department of Agriculture.
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as the plan is approved and when it has been determined that the last animals will be removed or the ownership of those animals transferred. CSU may elect to maintain the pens on its property or request that the ground be returned to its original state by Veterinary Services.

## NOTIFICATIONS

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On acceptance of the plan, several notifications will take place.

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- Dr. Jack Rhyan will notify collaborators.
- Dr. Don Herriott will notify the NPS.
- Dr. Shere will provide any notifications necessary for VS.

Suelee,

These are the main points I want you all to be aware of as you write the response.

General Comments:

In 2010 and 2011, as we were planning the GonaCon study in bison, I was in contact with our AgSAS person, Freeda Isaac, concerning the regs and how they were applied. In an email dated 4/02/2010, she informed me:

“Although naturally infected animals are not considered select agents themselves and not subject to the select agent regulations, once these animals are confirmed as positive for a select agent, any materials from these animals would be treated as select agent material. The infected cattle are considered the natural source of the Brucella and the materials from these animals are being intentionally collected. This is found in 9 CFR 121.3(d)(1). For example, blood, tissue specimens, urine, etc. would be subject to handling as select agent material. My understanding is these Idaho cattle have been confirmed for *B. abortus* by VS, therefore the materials from these cattle would need to be handled in accordance with the select agent requirements.”

Later the same year, in an email dated 5/13/2010, she told me of a change in the way the regs were now being interpreted:

“As we had discussed over the phone several weeks ago, the Select Agent Program directors were meeting to discuss the issues related to naturally infected versus experimentally infected animals and the status of samples taken from these animals.

In our discussions, it was agreed upon that for naturally affected animals, samples taken from those animals would not be considered select agent material and required to be handled as restricted material until the sample was confirmed to have select agent material. For the issues you have raised below for the cattle you have, the samples may be handled as you have described and not subject to select agent requirements until the sample itself is confirmed positive for select agents.”

We had initially planned to stockpile and freeze samples from the GonaCon study bison, so that the culture status of all the samples from the animals would remain unknown until the end of the study. However based on the 5/13/2010 email, and in a telephone consultation about the GonaCon study with Freeda Isaac on May 11, 2011, we were assured that we could sample seropositive naturally-infected animals time after time and submit diagnostic specimens to the lab for culture. We were assured that this field study, observing the disease in its natural environment, a bison population which contained both seropositive and seronegative animals, could be conducted in such a manner as to be fully compliant with the SA regs. That is what we did. We maintained naturally-infected animals and repeatedly obtained diagnostic specimens from these animals for culture. We shipped all diagnostic specimens for culture to the lab at once such that if any specimen from an animal was found culture positive, there was not an issue with other specimens from that animal being held. We never had possession of any known culture positive specimen or culture of *B. abortus* outside of the naturally-infected animal thus we were always in full compliance with the SA regs.

The elk study was commenced using undiagnosed elk fetuses only after consultation with AgSAS in February 2014. The fetuses were later submitted for culture. One was positive and one was negative. No natural (or experimental) transmission occurred.

Even according to the “Guidance on the Inventory of Select Agents and Toxins – 16 April 2015,” we were still in full compliance. We never experimentally infected any animals with *B. abortus*. We have placed seropositive animals in the same pen with seronegative animals to observe whether or not natural transmission would occur. This was identical to the 6 year study we did in Yellowstone National Park observing whether or not and when transmission would occur between seropositive and seronegative animals.

Only in the Policy Statement dated August 18, 2017, which is obviously written after the fact to address our work, is there language of which we would have been in violation. At the time this statement was released, we had already interrupted both bison and elk studies and were in the process of killing research animals.

We consulted with AgSAS about these studies and purposed to remain in full compliance with the SA regs, as they were explained to us. That is what we did. On learning that the new interpretation of the regs put our protocols in question, we ended both studies.

Inaccuracies in the “Advisory Letter on Violations of the Select Agent Regulations” to Bev Schmitt dated August 17, 2017.

2<sup>nd</sup> paragraph: No individual has knowingly possessed or worked with *Brucella abortus*. Individuals have collected specimens from seropositive, naturally-infected animals, some of which have later proven to be culture positive. This same situation occurs routinely in packing houses and field operations. Hence, these individuals were not required to have approval or be registered to possess or use *B. abortus*.

3<sup>rd</sup> paragraph: No person knowingly had possession of *B. abortus*. They only handled naturally-infected animals and obtained diagnostic specimens of unknown culture status.

4<sup>th</sup> paragraph: NVSL was observing whether or not natural transmission occurred between seropositive and seronegative animals.

2<sup>nd</sup> page, #1. The elk were not purchased from Yellowstone National Park. Commercial elk were purchased from a breeder in Colorado and elk from the Greater Yellowstone Area were wild-caught in Wyoming with the cooperation and permission of Wyoming Game and Fish.

Jack



Jan 8, 2012

	A	B	C	D	E	F	G	H	I	J	K	L
1	BANGLE TAG	EARTAG	Sero-stat	Age/DOB	SEX	Old Eartag	Bled	Vag. Swab	Preg?	Deworm	Implant	Sniff?
2	Green 08	YNP930648	NEG	4, 2009	F		✓	✓	✓		.183✓	✓
3	Green 09	YNP930755	NEG	3, 2010	F		✓	✓	✓		.702✓	✓
4	Green 10	YNP930626	NEG	4, 2009	F		✓	✓	✓		.542✓	✓
5	Green 14	YNP930725	NEG	4, 2009	F		✓	✓	✓	.052✓	.621✓	✓
6	Green 15	YNP930634	NEG	3, 2010	F		✓	✓	✓		.132✓	✓
7	Red 03	YNP930689	POS	4, 2009	F		✓	✓	✓		.162✓	✓
8	Red 06	YNP930287	POS	3, 2010	F		✓	✓	✓		.572✓	✓
9	Red 07	YNP930773	POS	4, 2009	F		✓	✓	✓		.193✓	✓
10	Red 08	YNP930761	POS	4, 2009	F		✓	✓	✓			✓
11	Red 09	YNP930760	POS	2, 2011	F		✓	✓	open			✓
12	Red 13	YNP930737	POS	3, 2010	F		✓	✓	✓	Warts	.692✓	✓
13	Red 15	YNP930706	POS	3, 2010	F		✓	✓	No : Alaskan?		<del>.333</del>	✓
14	Red 16	YNP930684	POS	3, 2010	F		✓	✓	✓		.333✓	✓
15	Red 17	YNP930588	POS	3, 2010	F		✓	✓	open			✓
16	Red 18	YNP930776	POS	4, 2009	F		✓	✓	✓		.752✓	✓
17	Red 21	YNP930763	POS	4, 2009	F		✓	✓	✓		.582✓	✓
18	Red 22	YNP930673	POS	4, 2009	F		✓	✓	✓		.301✓	✓
19	Red 25	YNP930778	POS	4, 2009	F		✓	✓	✓		.041✓	✓
20	Red 30	YNP930568	POS	3, 2010	F		✓	✓	✓		.633✓	✓
21												
22												
23												
24												
25												
26												
27												



Jan 9, 2012

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	BANGLE TAG	EARTAG	Sero-stat	Age/DOB	SEX	Old Eartag	BLED	Vag. Swab	Preg?	Disposition	Deworm	Implant	Sniff?
2	Green 02	YNP930702	NEG	3, 2010	F		✓	✓	✓	Frank's	✓	641✓	✓
3	Green 03	YNP930731	NEG	3, 2010	F		✓	✓	✓	Frank's	✓	551✓	✓
4	Green 04	YNP930625	NEG	4, 2009	F		✓	✓	open	Frank's	✓	—	✓
5	Green 06	YNP930754	NEG	3, 2010	F		✓	✓	open	Frank's	✓	—	✓
6	Green 17	YNP930627	NEG	4, 2009	F		✓	✓	✓	Frank's	✓	050✓	✓
7	Red 01	YNP930472	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
8	Red 02	YNP930705	SUS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
9	Red 04	YNP930759	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
10	Red 05	YNP930697	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
11	Red 11	YNP930777	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
12	Red 14	YNP930150	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
13	Red 19	YNP930762	POS	3, 2010	F		✓	✓	—	Frank's	✓	—	✓
14	Red 20	YNP930678	POS	4, 2009	F		✓	✓	✓	Frank's	✓	720✓	✓
15	Red 23	YNP930667	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
16	Red 24	YNP930636	POS	4, 2009	F		✓	✓	✓	Frank's	✓	801✓	✓
17	Red 26	YNP930202	POS	4, 2009	F		✓	✓	✓	Frank's	✓	662✓	✓
18	Red 27	YNP930454	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
19	Red 28	YNP930575	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
20	Red 29	YNP930406	POS	4, 2009	F		✓	✓	—	Frank's	✓	—	✓
21	Red 31	YNP930696	POS	3, 2010	F	Green 05	✓	✓	—	Frank's	✓	—	✓
22													
23													
24													
25													
26													
27													
28													

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6628

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](http://www.biotracking.com)

BioPRYN PSPB Report

Date Received	Log In #
2/4/2014	O20414001

Submitted By	Report To
USDA/APHIS/VS 4101 LaPorte Avenue Fort Collins, CO 80521	Dr. Jack Rhyan  <a href="mailto:jack.c.rhyan@aphis.usda.gov">jack.c.rhyan@aphis.usda.gov</a>

REPORT NOTES:  
Mail Report  
Tube numbers 47 through 52 also include "Bison" in the tube label.

Report Date	Assay/Animal	Number of Samples
02/05/2014	Bison - 52 sample(s)	52

Open	Low Recheck	Cutoff	High Recheck	Pregnant
OD < 0.135	OD = 0.135 to 0.15	0.15	OD = 0.15 to 0.21	OD > 0.21

Tube Number	Animal ID	Response in Test, OD	PSPB Range	Days Post Breeding
1	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

001050

20	R04	0.0559	Open	>40
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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BioTracking LLC

1150 Alturas Dr  
Suite 105  
Moscow, ID 83843  
Phone: 208.882.9736  
Fax:208.882.1490  
email: biotracking@turbonet.com  
web: [www.biotracking.com](http://www.biotracking.com)

BioPRYN PSPB Report

Date Received	Log In #
12/5/2013	120513009

Submitted By	Report To
USDA National Wildlife Research Center 4101 LaPorte Avenue Fort Collins, CO 80521	Jack Rhyan

[jack.c.rhyan@aphis.usda.gov](mailto:jack.c.rhyan@aphis.usda.gov)

REPORT NOTES:  
Mail Report

Report Date	Assay/Animal	Number of Samples
12/06/2013	Bison - 19 sample(s)	19

Open	Low Recheck	Cutoff	High Recheck	Pregnant
OD < 0.135	OD = 0.135 to 0.15	0.15	OD = 0.15 to 0.21	OD > 0.21

Tube Number	Animal ID	Response in Test, OD	PSPB Range	Days Post Breeding
1	Yellow 1	0.3697	Pregnant	40
2	Yellow 2	0.0639	Open	40
3	Yellow 3	0.0644	Open	40
4	Yellow 4	0.064	Open	40
5	Yellow 5	0.0583	Open	40
6	Yellow 6	0.0646	Open	40
7	Yellow 7	0.0692	Open	40
8	Yellow 8	0.0853	Open	40
9	Yellow 9	0.0798	Open	40
10	Yellow 10	0.07	Open	40
11	Green 11	0.0781	Open	40
12	Green 12	0.6185	Pregnant	40
13	Green 14	0.2884	Pregnant	40
14	Green 15	0.3439	Pregnant	40
15	Green 16	0.0772	Open	40
16	Green 17	0.5002	Pregnant	40
17	Green 18	0.4087	Pregnant	40
18	Green 19	0.4555	Pregnant	40
19	Green 20	0.0699	Open	40

BioPRYN measures the presence of Pregnancy-Specific Protein B (PSPB) in serum and the attached results are provided for your interpretation. If a sample's OD falls in the Open range, 99.9% of animals are not pregnant in

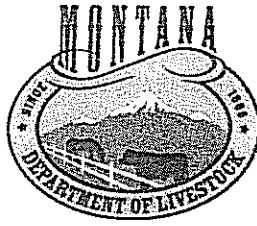
001052

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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STATE OF MONTANA

DEPARTMENT OF LIVESTOCK  
DIAGNOSTIC LABORATORY  
PO BOX 997  
BOZEMAN, MONTANA 59771



PHONE 406-994-4885  
FAX 406-994-6344  
E-MAIL [livdiagnosticlab@mt.gov](mailto:livdiagnosticlab@mt.gov)

DATE: 01-31-2017

TO: USDA / APHIS  
\_\_\_\_\_  
\_\_\_\_\_

EMAIL: \_\_\_\_\_ FAX: \_\_\_\_\_

FROM: Antonio Fuentes  
Serology

COMMENT: 17-10790

Previous report sent by e-mail had  
an error on FPA results  
See corrections

Have a good day,  


Total number of pages including this cover sheet: 4

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Monday, April 20, 2015 12:22 PM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. Classif.

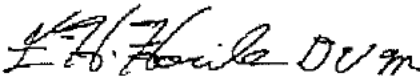
On 4/20/2015 10:27 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Would you be able to give us a final classification for this animal.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: Case#8-372-15, ID- R06 Adult Fe. Bison. This animal is classified as a reactor based on positive

  
Designated Brucellosis Epidemiologist

FP serology. Regards Frank Houle



## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) <[REDACTED]@gmail.com>  
**Sent:** Friday, May 08, 2015 11:15 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. Classification

On 5/8/2015 10:44 AM, Fuentes, Antonio wrote:

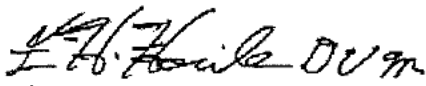
Greetings Dr. Houle,

Hope you are doing well.

Would you be able to send a final classification for GonaCon Study bison.

Thanks,  
Antonio

Hi Antonio: Case# 8-385-15 Gona Con Study Dr. Ryan Clarke. Animals ID#s 5G15,R14, G15 &5R14 are classified as Reactors. Animal ID# 5G03 is classified as a suspect. Note the calf reactions may be colostrum

  
Designated Brucellosis Epidemiologist

related. Regards and Thanks Frank

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Monday, May 18, 2015 10:36 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. classification

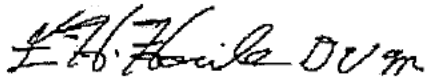
On 5/18/2015 8:32 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Need a final classification for these four bison from the GonaCon study.  
Thanks.

Have a good day,  
Antonio

Hi again Antonio: Case# 8-401-15, Dr.Ryan Clarke, Gona Con Study  
Tube# 2 ID R09 is classified as a reactor based on positive serological reactions. Regards Frank

  
Designated Brucellosis Epidemiologist

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Wednesday, May 13, 2015 11:25 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. classification

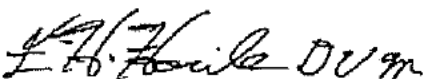
On 5/13/2015 9:23 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

See attached report with testing result.  
Would you give us a final classification?  
Thanks.

Have a good day,  
Antonio

Good Morning Antonio: Case#8-388-15 Gona Con Study Con study Dr. Ryan Clarke ID's #5R26,R26,5R18,R18,5G09 and G09 are classified as reactors based on serological reactions. Regards



Designated Brucellosis Epidemiologist

Frank

## Fuentes, Antonio

---


**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Wednesday, May 13, 2015 11:41 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Bru fiant classification

On 5/13/2015 9:56 AM, Fuentes, Antonio wrote:

Oops! I guess it did not attached properly.  
Let's try again...

This one worked.

Hi Antonio: Case# 8-398-15 Gona Con study Dr. Ryan Clarke ID's # R13 and 5R13 are classified as reactors based on FP serological reactions. #s 5G08 and 5G04 are classified as suspects based on CF reactions (the CF reactions probably represent nonspecific background) Regards Frank

  
Designated Brucellosis Epidemiologist

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Thursday, June 18, 2015 11:31 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final bru classif.

On 6/18/2015 10:50 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Hope you are enjoying this nice weather.

Would you be able to give us a final classification for two GonaCon Study bison charts.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: Gona Con Study Dr. Ryan Clarke Case#8-439-15. The following are classified as reactors based on positive serological reactions.  
Tubes#s 2 R45,#3 R39,#4 R47,#6 R54,#7 R48, #12 R38,,#15 R53, #17R42,#17 R42,#18 R41,#19 R56,#20 R34,#25 R44, #26 R36, #27, R55, #28 R43, #29 R49,#30 R46, &#31 R51  
Tubes#s 13 Gr 19 & #14 GR 29 are classified as suspects.

Case#8-455-15, Tubes#s 2 R04, #5 R19, #6 R27, #7 R28, #8 R29 are classified as reactors. Tube# 1 R01 is classified as a suspect.

Regards Frank

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Thursday, June 18, 2015 11:31 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final bru classif.

On 6/18/2015 10:50 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Hope you are enjoying this nice weather.

Would you be able to give us a final classification for two GonaCon Study bison charts.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: Gona Con Study Dr. Ryan Clarke Case#8-439-15. The following are classified as reactors based on positive serological reactions.  
Tubes#s 2 R45,#3 R39,#4 R47,#6 R54,#7 R48, #12 R38,,#15 R53, #17R42,#17 R42,#18 R41,#19 R56,#20 R34,#25 R44, #26 R36, #27, R55, #28 R43, #29 R49,#30 R46, &#31 R51  
Tubes#s 13 Gr 19 & #14 GR 29 are classified as suspects.

Case#8-455-15, Tubes#s 2 R04, #5 R19, #6 R27, #7 R28, #8 R29 are classified as reactors. Tube# 1 R01 is classified as a suspect.

Regards Frank

**Fuentes, Antonio**

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Friday, June 19, 2015 10:44 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final classif. Case No. 8-452-15

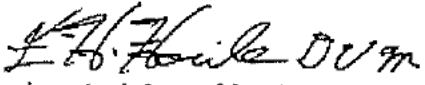
On 6/18/2015 4:01 PM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Would you give us a final classif. For this bison from GonaCon Std.?

Thanks.  
Antonio

Hi Antonio: Case#8-452-15, GonaCon study ,Dr. Ryan Clarke: All six animals are classified Reactors based on



Designated Brucellosis Epidemiologist

positive serological reactions. Regards

Frank



# MVDL

## MONTANA VETERINARY DIAGNOSTIC LABORATORY

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

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

Accession #: 8-397-15

Owner: USDA/APHIS/VS

Species: WILD - BISON

Breed: BISON

Name/No. 5G07

Age: NEWB(Sex:

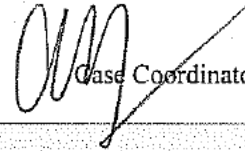
Date Sent: 05/13/2015

Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

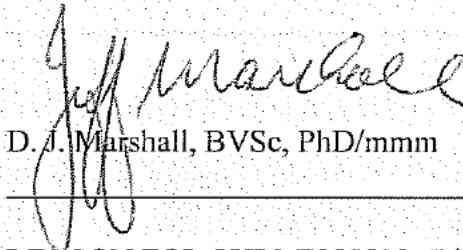
### Final Report

  
Case Coordinator: JM

### CASE SUMMARY

CORRECTED REPORT 5/13/15:

BACTERIOLOGY: I commented that tissues from this calf tested positive for Brucella when in fact the bacteriology report stated otherwise. The bacteriology report is correct. Brucella was not isolated from this bison calf.

  
D. J. Marshall, BVSc, PhD/mmm

REASON FOR SUBMISSION: Bison calf abortion

#### LABORATORY DIAGNOSIS:

Bison calf abortion; Brucella abortus

COMMENT: Brucella cultures for this case were inconclusive due to Proteus overgrowth.

D. J. Marshall, BVSc, PhD\cto

Date In: 05/04/2015

### PATHOLOGY

Date Out: 05/11/2015

Released by: JM

GROSS PATHOLOGY: A bison calf (ID 5G07) was submitted for necropsy. Necropsy is performed at 10.30 am 4th May 2015. Calf is autolyzed and predated. Male calf had a crown rump length measurement of 91 cm. Abdominal organs are missing. Brain was not examined.

HISTOPATHOLOGY: Sections of brain, lung, skeletal muscle and thymus. Tissues are moderately autolyzed. Lung is not aerated and alveoli and airways contain quantities of meconium and squamous epithelial debris.

#### MORPHOLOGIC DIAGNOSIS:

Lung: Non-aeration; Intra-alveolar meconium and squamous epithelial debris and meconium

Date In: 05/04/2015

### BACTERIOLOGY

Date Out: 05/11/2015

Released by: mh



MVDL Accession #:  
8-397-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA/APHIS/VS

Date In: 05/04/2015

Date Out: 05/11/2015 Released by: mh

Brucella culture results inconclusive due to Proteus overgrowth.

**CULTURES**

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	3+	NA
	fetal lung	Brucella	Proteus overgrowth		NA

1+ to 4+ = rare colony to confluent growth

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

MVDL Accession #:  
8-397-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA/APHIS/VS

## Fees

Bacteriology Fee	\$ 45.00
Pathology/Histology Fee	\$ 35.75
Accession Total Fee	\$ 80.75

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



# 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 # 8-396-15

Owner: USDA/APHIS/VS

Species: WILD - BISON

Breed: BISON

Name/No. 5R09

Age: NEWB(Sex:

Date Sent: 05/15/2015

Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

### Final Report

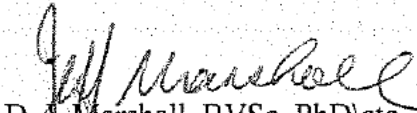
Case Coordinator: JM

### CASE SUMMARY

5/15/15

#### ADDITIONAL INFORMATION:

**BACTERIOLOGY:** The Brucella isolate was confirmed as Brucella abortus by identification testing at NVSL (see attached report).

  
D. J. Marshall, BVSc, PhD\cto

#### ADDITIONAL INFORMATION 5/13/15:

**BACTERIOLOGY:** Brucella sp was isolated from this calf. The isolate has been forwarded to NVSL for further identification procedures. Results will be forwarded as soon as available.

D. J. Marshall, BVSc, PhD/mmm

5/11/15

**REASON FOR SUBMISSION:** Bison calf abortion

#### LABORATORY DIAGNOSIS:

Bison calf abortion

**COMMENT:** Results of bacteriological investigations will be reported as soon as complete.

D. J. Marshall, BVSc, PhD\cto

Date In 05/04/2015

**PATHOLOGY**

Date Out: 05/11/2015

Released by: JM

**GROSS PATHOLOGY:** A bison calf (ID 5R09) was submitted for necropsy. Necropsy is performed at 11 am 4th May 2015. Calf is autolyzed and predated. Sex could not be determined. Crown rump length measured 76 cm. Only a small portion of brain, lung and skeletal muscle was available for examination. Brain was severely autolyzed and not sampled.

**HISTOPATHOLOGY:** Sections of lung and skeletal muscle are examined. Lung is severely autolyzed and not useful for diagnostic purposes. No significant abnormality is detected in skeletal muscle.

**MORPHOLOGIC DIAGNOSIS:**

Lung: Autolysis

Date In 05/04/2015

**BACTERIOLOGY**

Date Out: 05/15/2015

Released by: mh

Isolate to be sent to NVSL for full identification 5/12/15.

**CULTURES**

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	2+	NA
	fetal lung	Brucella	Brucella abortus	3+ M	NA

1+ to 4+ = rare colony to confluent growth

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

Date In: 05/11/2015

**REFERRAL/OTHER**

Date Out: 05/15/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
5R09	Slant Tube	Brucella Culture	See attached report	NVSL

Please see attached report for complete results.

**National Veterinary Services Laboratories**

PO Box 844

Ames, Iowa 50010

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

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FINAL REPORT

**Laboratory Test Report**

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

**Owner**

USDA, APHIS, VS

Corwin Springs, MT

**Animal Location**

Park County MT

**Submitter - 2047**

MT Department of Livestock

Diagnostic Laboratory Division

1911 W Lincoln St

PO Box 997

Bozeman, MT 59718

FAX #: 406-994-6344

Phone #: 406-994-4885

**Accession Number:****15-015494****Date Collected:**

05/02/2015

**Date Received:**

05/13/2015

**Date Completed:**

05/15/2015

**Collected By:**

Dr. Patrick Ryan Clarke

**Purpose:**

General Diagnostic

**Referral Number:**

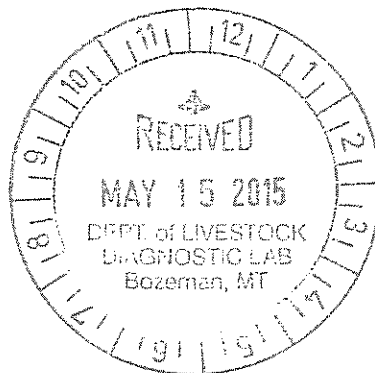
8-396-15

**This is not a billable case.****NOTE: Condition of the sample(s) was adequate unless otherwise noted.**

Sample: 8-396-15 Animal ID: 5R09 Brucella Case Number: B15-0160 Specimen Type: Culture Species: Bison

Brucella Final Identification

Brucella abortus

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

Scanned 5-15-15

---

**Help Us Help You**

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

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

## Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 73.50
Referral Fee	\$ 19.10
Accession Total Fee	\$ 92.60

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

**Seronegative animals :**

Removed from research pens to start quarantine process for conservation release. All or a portion may be transferred to Colorado State University or the State of MT DOL for this purpose.

**Seropositive Treatment cows:**

After final calving year, will be euthanized and tissues collected for final culture data. Carcass will be buried in landfill.

**Seropositive non-treatment and other seropositive animals:**

Will be shipped to slaughter with tissues collected for final culture data. Carcass will be made available to the MT Food Bank.

**Timeline (if MT DOL or another entity were able to take over the project):**

The **first cohort of control animals** are due to be removed from the study in January 2018. None of the animals were given GonaCon, all are seropositive and will be shipped to slaughter where tissues will be collected. All seronegative offspring will be quarantined for conservation and may be transferred as above for that purpose.

The **first cohort of treatment animals** have failed to all become pregnant, meaning that they have yet to complete the study. All negative cows could be removed, all positive cows that have calved will be euthanized and tissues collected January 2018 and all non-pregnant treatment cows will be added to second treatment cohort until they calve.

The **second cohort of control animals** will calve 3 more years before ending the study in winter of 2020.

The **second cohort of treatment animals** will calve 2 years before ending the study in winter of 2019.



## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Monday, April 20, 2015 12:22 PM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. Classif.

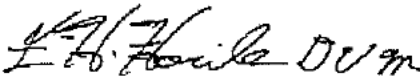
On 4/20/2015 10:27 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Would you be able to give us a final classification for this animal.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: Case#8-372-15, ID- R06 Adult Fe. Bison. This animal is classified as a reactor based on positive

  
Designated Brucellosis Epidemiologist

FP serology. Regards Frank Houle

## Fuentes, Antonio

---


**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Wednesday, May 13, 2015 11:41 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Bru fiant classification

On 5/13/2015 9:56 AM, Fuentes, Antonio wrote:

Oops! I guess it did not attached properly.  
Let's try again...

This one worked.

Hi Antonio: Case# 8-398-15 Gona Con study Dr. Ryan Clarke ID's # R13 and 5R13 are classified as reactors based on FP serological reactions. #s 5G08 and 5G04 are classified as suspects based on CF reactions (the CF reactions probably represent nonspecific background) Regards Frank

  
Designated Brucellosis Epidemiologist

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Wednesday, May 13, 2015 11:25 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. classification

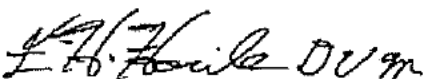
On 5/13/2015 9:23 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

See attached report with testing result.  
Would you give us a final classification?  
Thanks.

Have a good day,  
Antonio

Good Morning Antonio: Case#8-388-15 Gona Con Study Con study Dr. Ryan Clarke ID's #5R26,R26,5R18,R18,5G09 and G09 are classified as reactors based on serological reactions. Regards



Designated Brucellosis Epidemiologist

Frank

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Monday, May 18, 2015 10:36 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. classification

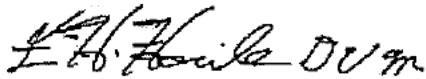
On 5/18/2015 8:32 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Need a final classification for these four bison from the GonaCon study.  
Thanks.

Have a good day,  
Antonio

Hi again Antonio: Case# 8-401-15, Dr.Ryan Clarke, Gona Con Study  
Tube# 2 ID R09 is classified as a reactor based on positive serological reactions. Regards Frank

  
Designated Brucellosis Epidemiologist

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Thursday, May 28, 2015 1:59 PM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. Classification

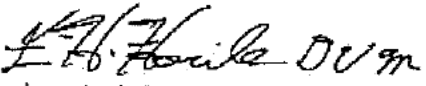
On 5/28/2015 1:20 PM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

See attached chart for final brucellosis classification.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: GonaCon study case#8-403-15 Dr. Ryan Clarke. ID# R24 is classified as a reactor based on FP results. 5R07 (calf) is classified as a reactor based on CF results (this CF reaction may be a non specific

  
Designated Brucellosis Epidemiologist

background reaction) Regards Frank



# MVDL

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

Accession # 8-413-15

Owner: USDA, APHIS, VS

Submitter: PATRICK RYAN CLARKE D.V.M.

Species: WILD - BISON

Breed: BISON

Name/No. G10

Age: ADULT Sex: F

Date Received 05/18/2015

Preliminary Date 05/26/2015

On  
VMD

(b) (6)

### Preliminary Report

Case Coordinator: JM

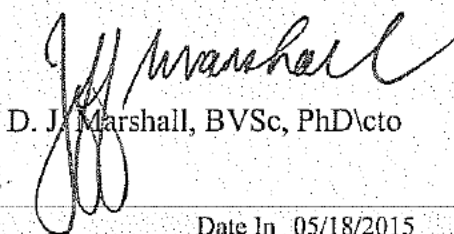
### CASE SUMMARY

REASON FOR SUBMISSION: Bison abortion

#### LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.

  
D. J. Marshall, BVSc, PhD\cto

Date In 05/18/2015

### PATHOLOGY

Date Out: 05/26/2015

Released by: JM

GROSS PATHOLOGY: A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

HISTOPATHOLOGY: Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

#### MORPHOLOGIC DIAGNOSIS:

Placenta: Placentitis, necrotizing, severe

Date In 05/19/2015

### BACTERIOLOGY

Date Out:

Released by:

#### CULTURES

Antimicrobial  
Growth Profile

ID/Site	Specimen	Culture Type	Isolate	Growth	Profile
	placenta	Brucella	Results Pending		
	lung	Brucella	Results Pending		
	abomasal swab	Brucella	Results Pending		

MVDL Accession #  
8-413-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA, APHIS, VS

Date In: 05/19/2015		REFERRAL/OTHER		Date Out:	Released by: JM
<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>	
	Tissue	Brucella Culture		NVSL	
Results Pending					



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

Accession #: 8-413-15

Owner: USDA, APHIS, VS

Species: WILD - BISON

Breed: BISON

Date Received: 05/18/2015

Name/No. G10

Preliminary Date 06/01/2015

Submitter: PATRICK RYAN CLARKE D.V.M.

Age: ADULT Sex: F

(b) (6)

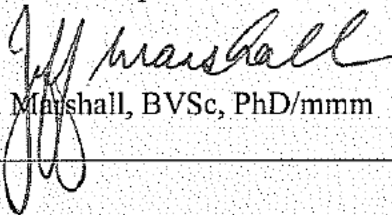
### Preliminary Report

Case Coordinator: JM

### CASE SUMMARY

ADDITIONAL INFORMATION 6/1/15:

NVSL: Attempts to culture Brucella sp from the tissues submitted from the dam were negative.  
See attached report.

  
D. J. Marshall, BVSc, PhD/mmm

REASON FOR SUBMISSION: Bison abortion

#### LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.

D. J. Marshall, BVSc, PhD\cto

Date In 05/18/2015

### PATHOLOGY

Date Out: 05/26/2015

Released by: JM

GROSS PATHOLOGY: A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

HISTOPATHOLOGY: Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

#### MORPHOLOGIC DIAGNOSIS:

Placenta: Placentitis, necrotizing, severe

001122



MVDL Accession #:  
8-413-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA, APHIS, VS

Date In: 05/19/2015

## BACTERIOLOGY

Date Out:

Released by:

### CULTURES

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	placenta	Brucella	Results Pending		
	lung	Brucella	Results Pending		
	abomasal swab	Brucella	Results Pending		

Date In: 05/19/2015

## REFERRAL/OTHER

Date Out: 06/01/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
G10	Tissue	Brucella Culture	No isolation made	NVSL

Please see attached report for complete results.



## National Veterinary Services Laboratories

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Ames, Iowa 50010

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

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FINAL REPORT

## Laboratory Test Report

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

## Owner

USDA,APHIS,VS  
Corwin Springs, MT

Accession Number: 15-016456

## Animal Location

Park County MT

Date Collected: 05/18/2015

Date Received: 05/20/2015

## Submitter - 2046

MT Department of Livestock  
Diagnostic Laboratory Division  
1911 W Lincoln St  
PO Box 997  
Bozeman, MT 59718  
FAX #: 406-994-6344  
Phone #: 406-994-4885

Date Completed: 06/01/2015

Collected By: Dr. P. Ryan Clarke

Purpose: General Diagnostic

Referral Number: 8-413-15

This is not a billable case.

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

Sample: 8-413-15 Animal ID: G10 Brucella Case Number: B15-0162 Specimen Type: Tissue Species: Bison

Brucella Isolation Result

No Isolation Made

Individual specimen results are listed below:

## Tissue / Lymph Node- S. Mammary

Brucella Isolation Result

No Isolation Made

## Tissue / Lymph Node- Retropharyngeal

Brucella Isolation Result

No Isolation Made

## Tissue / Lymph Node- Popliteal

Brucella Isolation Result

No Isolation Made

## Tissue / Lymph Node- Mandibular

Brucella Isolation Result

No Isolation Made

## Tissue / Lymph Node- Parotid

Brucella Isolation Result

No Isolation Made

## Tissue / Spleen

Brucella Isolation Result

No Isolation Made

## Tissue / Mammary Gland

Brucella Isolation Result

No Isolation Made

## Tissue / Kidney

Brucella Isolation Result

No Isolation Made

## Tissue / Lung

Brucella Isolation Result

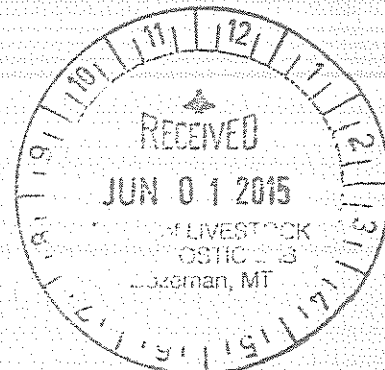
No Isolation Made

## Tissue / Mesentery

Brucella Isolation Result

No Isolation Made

Most samples had heavy contamination.



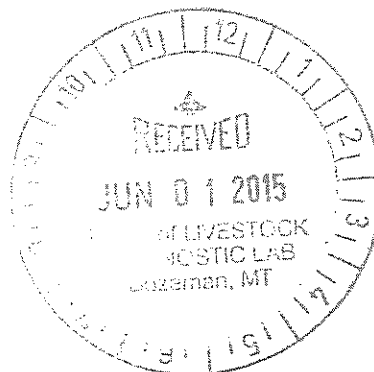
Scanned 6-1-15/jm

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

Help Us Help You

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

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





# MVDL

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

Accession # 8-413-15

Owner: USDA, APHIS, VS

Submitter: PATRICK RYAN CLARKE D.V.M.

Species: WILD - BISON

Breed: BISON

Name/No. G10

Age: ADULT Sex: F

Date Sent:

Date Received 05/18/2015

Preliminary Date 06/02/2015

(b) (6)

### Preliminary Report

Case Coordinator: JM

### CASE SUMMARY

#### 6-02-15 ADDITIONAL INFORMATION:

CLINICAL MICROBIOLOGY: Brucella sp was not isolated from fetal tissue cultures (see attached report).

COMMENT: I will further investigate placenta and the cause of histological changes present.

*D. J. Marshall*  
D. J. Marshall, BVSc, PhD/jmm

#### ADDITIONAL INFORMATION 6/1/15:

NVSL: Attempts to culture Brucella sp from the tissues submitted from the dam were negative. See attached report.

D. J. Marshall, BVSc, PhD/jmm

REASON FOR SUBMISSION: Bison abortion

#### LABORATORY DIAGNOSIS:

Bison abortion; Placentitis

COMMENT: Tissues from the cow were submitted to NVSL for bacteriological investigations. Bacteriological investigations of fetal tissues and placenta have been set up at this laboratory and will be reported as soon as complete.

D. J. Marshall, BVSc, PhD/cto

JL Accession #

8-413-15

Submitter:

PATRICK RYAN CLARKE D.V.M.

Owner:

USDA, APHIS, VS

Date In 05/18/2015

**PATHOLOGY**

Date Out:

Released by: JM

**GROSS PATHOLOGY:** A male bison fetus wrapped in placenta was submitted for necropsy and subsequent laboratory evaluation. The fetus has a crown rump measurement of 94 cm. There is generalized and marked autolysis and emphysema. Brain was not examined.

**HISTOPATHOLOGY:** Sections of placenta, liver, kidney, heart, lung, spleen, thymus, skeletal muscle, abomasum and ileum are examined. Placental surface is inflamed and necrotic with thick colonies of intralesional bacteria. Fetal tissues are moderately autolyzed. No significant histological abnormality is detected in these tissues.

**MORPHOLOGIC DIAGNOSIS:**

Placenta: Placentitis, necrotizing, severe

Date In 05/19/2015

**BACTERIOLOGY**

Date Out: 06/01/2015 Released by: mh

**CULTURES**

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	lung	Brucella	Negative for Brucella sp.		NA
	abomasal swab	Brucella	Negative for Brucella sp.		NA
	placenta	Brucella	Negative for Brucella sp.		NA

Date In: 05/19/2015

**REFERRAL/OTHER**

Date Out: 06/01/2015 Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrl Inst.</u>
119	Tissue	Brucella Culture	No isolation made.	NVSL

Please see attached report for complete results.



# MVDL

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

Accession # 8-396-15

Owner: USDA/APHIS/VS

Species: WILD - BISON

Breed: BISON

Name/No. 5R09

Age: NEWB(Sex:

Date Sent: 05/15/2015

Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

### Final Report

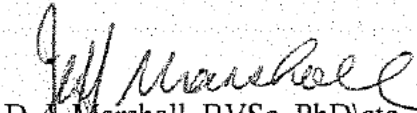
Case Coordinator: JM

### CASE SUMMARY

5/15/15

#### ADDITIONAL INFORMATION:

**BACTERIOLOGY:** The Brucella isolate was confirmed as Brucella abortus by identification testing at NVSL (see attached report).

  
D. J. Marshall, BVSc, PhD\cto

#### ADDITIONAL INFORMATION 5/13/15:

**BACTERIOLOGY:** Brucella sp was isolated from this calf. The isolate has been forwarded to NVSL for further identification procedures. Results will be forwarded as soon as available.

D. J. Marshall, BVSc, PhD\mmm

5/11/15

**REASON FOR SUBMISSION:** Bison calf abortion

#### LABORATORY DIAGNOSIS:

Bison calf abortion

**COMMENT:** Results of bacteriological investigations will be reported as soon as complete.

D. J. Marshall, BVSc, PhD\cto

Date In 05/04/2015

**PATHOLOGY**

Date Out: 05/11/2015

Released by: JM

**GROSS PATHOLOGY:** A bison calf (ID 5R09) was submitted for necropsy. Necropsy is performed at 11 am 4th May 2015. Calf is autolyzed and predated. Sex could not be determined. Crown rump length measured 76 cm. Only a small portion of brain, lung and skeletal muscle was available for examination. Brain was severely autolyzed and not sampled.

**HISTOPATHOLOGY:** Sections of lung and skeletal muscle are examined. Lung is severely autolyzed and not useful for diagnostic purposes. No significant abnormality is detected in skeletal muscle.

**MORPHOLOGIC DIAGNOSIS:**

Lung: Autolysis

Date In 05/04/2015

**BACTERIOLOGY**

Date Out: 05/15/2015

Released by: mh

Isolate to be sent to NVSL for full identification 5/12/15.

**CULTURES**

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	2+	NA
	fetal lung	Brucella	Brucella abortus	3+ M	NA

1+ to 4+ = rare colony to confluent growth

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

Date In: 05/11/2015

**REFERRAL/OTHER**

Date Out: 05/15/2015

Released by: JM

<u>Animal ID</u>	<u>Specimen</u>	<u>Test</u>	<u>Result</u>	<u>Rfrrl Inst.</u>
5R09	Slant Tube	Brucella Culture	See attached report	NVSL

Please see attached report for complete results.



# National Veterinary Services Laboratories

PO Box 844

Ames, Iowa 50010

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

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

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FINAL REPORT

## Laboratory Test Report

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

### Owner

USDA, APHIS, VS  
Corwin Springs, MT

### Animal Location

Park County MT

### Submitter - 2047

MT Department of Livestock  
Diagnostic Laboratory Division  
1911 W Lincoln St  
PO Box 997  
Bozeman, MT 59718  
FAX #: 406-994-6344  
Phone #: 406-994-4885

### Accession Number:

15-015494

### Date Collected:

05/02/2015

### Date Received:

05/13/2015

### Date Completed:

05/15/2015

### Collected By:

Dr. Patrick Ryan Clarke

### Purpose:

General Diagnostic

### Referral Number:

8-396-15

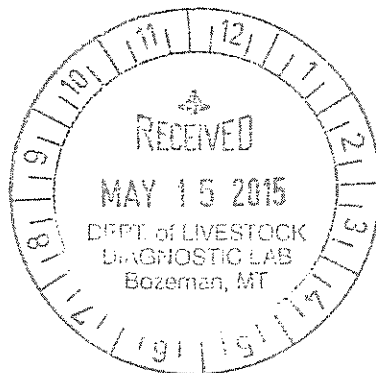
This is not a billable case.

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

Sample: 8-396-15 Animal ID: 5R09 Brucella Case Number: B15-0160 Specimen Type: Culture Species: Bison

Brucella Final Identification

Brucella abortus



### Results authorized by:

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

Scanned 5-15-15



---

**Help Us Help You**

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

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

## Fees

Bacteriology Fee	\$ 0.00
Pathology/Histology Fee	\$ 73.50
Referral Fee	\$ 19.10
Accession Total Fee	\$ 92.60

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



# MVDL

## MONTANA VETERINARY DIAGNOSTIC LABORATORY

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

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

Accession #: 8-397-15

Owner: USDA/APHIS/VS

Species: WILD - BISON

Breed: BISON

Name/No. 5G07

Age: NEWB(Sex:

Date Sent: 05/13/2015

Date Received: 05/04/2015

Submitter: PATRICK RYAN CLARKE D.V.M.

(b) (6)

### Final Report

*JM*  
Case Coordinator: JM

### CASE SUMMARY

CORRECTED REPORT 5/13/15:

BACTERIOLOGY: I commented that tissues from this calf tested positive for Brucella when in fact the bacteriology report stated otherwise. The bacteriology report is correct. Brucella was not isolated from this bison calf.

*D. J. Marshall*  
D. J. Marshall, BVSc, PhD/mmm

REASON FOR SUBMISSION: Bison calf abortion

LABORATORY DIAGNOSIS:

Bison calf abortion; Brucella abortus

COMMENT: Brucella cultures for this case were inconclusive due to Proteus overgrowth.

D. J. Marshall, BVSc, PhD\cto

Date In: 05/04/2015

### PATHOLOGY

Date Out: 05/11/2015

Released by: JM

GROSS PATHOLOGY: A bison calf (ID 5G07) was submitted for necropsy. Necropsy is performed at 10.30 am 4th May 2015. Calf is autolyzed and predated. Male calf had a crown rump length measurement of 91 cm. Abdominal organs are missing. Brain was not examined.

HISTOPATHOLOGY: Sections of brain, lung, skeletal muscle and thymus. Tissues are moderately autolyzed. Lung is not aerated and alveoli and airways contain quantities of meconium and squamous epithelial debris.

MORPHOLOGIC DIAGNOSIS:

Lung: Non-aeration; Intra-alveolar meconium and squamous epithelial debris and meconium

Date In: 05/04/2015

### BACTERIOLOGY

Date Out: 05/11/2015

Released by: mh

MVDL Accession #:  
8-397-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA/APHIS/VS

Date In: 05/04/2015

Date Out: 05/11/2015 Released by: mh

Brucella culture results inconclusive due to Proteus overgrowth.

**CULTURES**

<u>ID/Site</u>	<u>Specimen</u>	<u>Culture Type</u>	<u>Isolate</u>	<u>Growth</u>	<u>Antimicrobial Profile</u>
	fetal lung	Campylobacter	Negative for Campylobacter sp.		NA
	fetal lung	Aerobic	A mixed culture of non-pathogenic bacteria	3+	NA
	fetal lung	Brucella	Proteus overgrowth		NA

1+ to 4+ = rare colony to confluent growth

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

MVDL Accession #:  
8-397-15

Submitter:  
PATRICK RYAN CLARKE D.V.M.

Owner:  
USDA/APHIS/VS

## Fees

Bacteriology Fee	\$ 45.00
Pathology/Histology Fee	\$ 35.75
Accession Total Fee	<u>\$ 80.75</u>

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

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) <[REDACTED]@gmail.com>  
**Sent:** Friday, May 08, 2015 11:15 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final Bru. Classification

On 5/8/2015 10:44 AM, Fuentes, Antonio wrote:

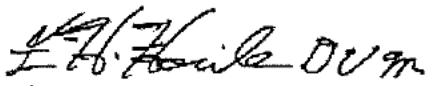
Greetings Dr. Houle,

Hope you are doing well.

Would you be able to send a final classification for GonaCon Study bison.

Thanks,  
Antonio

Hi Antonio: Case# 8-385-15 Gona Con Study Dr. Ryan Clarke. Animals ID#s 5G15,R14, G15 &5R14 are classified as Reactors. Animal ID# 5G03 is classified as a suspect. Note the calf reactions may be colostrum

  
Designated Brucellosis Epidemiologist

related. Regards and Thanks Frank

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

**Portfolio**  
**Wildlife/Livestock Disease Investigations Team**  
**2008**

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**Wildlife/Livestock Disease Investigations Team (WiLDIT)**  
**(Located at the National Wildlife Research Center, Fort Collins, CO)**

**Administrative History**

In 1997, following the '96/97 winter when over 1000 bison from Yellowstone National Park died or were sent to slaughter, Dr. Arnoldi, Deputy Administrator of APHIS/VS created the first position of the WiLDIT under the Western Regional Director, Dr. Bob Nervig. The position was located at the NWRC as part of the "One APHIS" concept. The purpose of the position was to help with GYA wildlife issues including continuing research projects begun in 1995. In 1999, when then Regional Director, Dr. Bill Buish, went to NVSL, Dr. Arnoldi placed the WiLDIT position under the VS Deputy's supervision and expanded the duties to include engagement in wildlife/domestic animal interface and gamefarm disease issues in which VS was involved. In 2000, Dr. Alfonso Torres replaced Dr. Arnoldi and reorganized his staff. He placed the position under Dr. Mike Gilsdorf of NCAHP. In 2001, Dr. Torres approved the WiLDIT to begin work on FMD in North American wildlife. In 2007 with Dr. Gilsdorf's retirement, NAHP was reorganized and the WiLDIT was placed under Dr. Diemer, the Assistant Regional Director of the Western Region and leader of the GYA core team.

**Staff**

Jack Rhyan DVM, MS - VMO

Matt McCollum - Wildlife Biologist

Pauline Nol DVM, MS - VMO; Attending Veterinarian for APHI/APHIS  
wildlife facility

Karl Held - Animal Health Technician, APHI/APHIS wildlife facility

Leah Swanekamp - Animal Health Technician, Bison Quarantine Feasibility  
Study in Montana, intermittent

Franklin Rigler - Animal Health Technician, Bison Quarantine Feasibility Study  
in Montana, part-time

Sarah Coburn, MS - Veterinary student on Saul Wilson Scholarship;  
scholarship provides part-time employment

Jon Pilon PhD - APHIS Science Fellow- shared with WS - administratively under  
WS

**Mission**

The mission of the WiLDIT is to serve VS in the area of wildlife/livestock interface diseases:

- Serve with the GYA Core Team to promote and achieve the elimination of brucellosis from GYA bison and elk

- provide and disseminate knowledge of diseases at the wildlife/domestic animal interface to APHIS/VS and others
- liaise with state and federal agencies including wildlife and gamefarm authorities and NGOs.
- develop science-based solutions to disease problems at the wildlife/domestic animal interface.

### **Activity Areas**

**1. Consultation** - Provide advice and consultation to Agency on interface disease issues; serve as liaisons with State and Federal wildlife agencies and NGO's. Serve as liaisons with WS.

**2. Developmental work** - Coordinate and/or conduct developmental work to address VS-specific problem areas, i.e. vaccine development for wildlife (brucellosis, TB, CWD, FMD), test diagnostics for wildlife (i.e., fluorescence polarization assay, infrared imaging technology), strategies to eliminate brucellosis from GYA wildlife (i.e., oral vaccination, immunocontraception, therapeutic vaccination, sustained release antibiotics). Developmental work is routinely done in collaboration with ARS, NWRC, and/or other federal agencies, CSU, and/or State wildlife agencies.

**3. Monitoring/Surveillance** - Conduct surveillance of wildlife around disease outbreaks or on continuing basis around endemic diseases, i.e. survey wildlife for TB around infected premises.

**4. Training** - Serve as training resource for the agency concerning interface diseases, i.e. trained contingent of VMOs on wildlife diseases in 2002. Serve as wildlife disease instructors at *Brucella* epi courses, FAD courses, CSU courses, and other Federal and State agency animal disease courses.

### **Funding**

APHIS funding for WiLDIT has been primarily from the brucellosis account. Additionally, depending on the projects conducted, some funds have been transferred from TB funds, and from end-of-year money. Some projects have been funded, in part, by our collaborators from grants received from NSCREES, CSU, and NPS. Following are records of obligations for the past 5 years. These do not include salaries for Jack Rhyan and Matt McCollum.

	2004	2005	2006	2007	2008*
Salaries/benefits/overtime	26,000	52,000	67,500	147,000	201,000
Travel and transport	31,000	22,500	30,000	13,000	50,000
Rent/commun/utilities	50,000	50,000	80,000	74,000	50,000
Services	11,500	41,000	80,000	20,000	10,000
Supplies (scientific, feed, etc)	113,000	89,000	75,000	73,000	80,000
Equipment	27,500	3,000	8,500	3,000	0
Other	0	4,000	1,000	0	0
<b>Total obligations</b>	<b>259,000</b>	<b>261,500</b>	<b>342,000</b>	<b>330,000</b>	<b>391,000</b>

\*Projected spending

### Justifications

**Why in Veterinary Services?** In 1997, when the first position was established, Wildlife Services was not involved in any disease work with the exception of rabies. Since then, WS has developed a disease program including work on AI, TB, CWD, and continuing work in rabies. The VS Team has consistently liaised with WS in the development of the WS work and routinely collaborates with WS personnel in laboratory research and field work. This arrangement works well for both sister agencies. Examples of collaborative work include: CWD vaccine developmental work, immunocontraceptive development, sustained release antibiotic development, FLIR technology for animal disease surveillance, *Brucella* wildlife vaccine development, TB wildlife vaccine development, etc.

**Why in Fort Collins?** The Team's location at the NWRC is beneficial to both VS and WS. Additionally, the Fort Collins location allows frequent collaboration with other VS, ARS, CSU, DOI, and State domestic animal and wildlife disease experts. A valuable continuing relationship for the WiLDIT is that of the Animal Population Health Institute at CSU. The two entities share a wildlife disease research facility and routinely collaborate on projects. The CSU connection has been valuable in providing funding and students for needed disease studies. In addition, APHI has necessary, essential, specialized laboratory resources that WiLDIT personnel have utilized on collaborative projects.

### Major Interface Disease Issues in which WiLDIT is Engaged

**1. Brucellosis in GYA wildlife and feral swine:** The primary disease issue the Team has been engaged in since its creation is the endemic infection of GYA bison and elk with *Brucella abortus*. The Team's involvement with this issue include: liaising with other involved agencies in several venues, collaborative research on the disease in wildlife and the risks posed to cattle, vaccine development for wildlife, serving as experts for agency epidemiologists in

development of risk assessments, cost-benefit analyses, decision memos, etc., and development of nonlethal strategies to eradicate the disease from GYA wildlife.

**2. TB in Michigan deer:** Team involvement in this issue includes wildlife surveillance around infected cattle premises, vaccine and vaccine marker development, bait development for vaccine delivery, and interagency planning of a future field trial in Michigan using the developed vaccine in deer.

**3. CWD in cervids:** Team involvement includes a 6 year study of CWD-exposed fallow deer and CWD vaccine development studies in mice and mule deer.

**4. FMD in North American wildlife:** WiLDIT personnel have collaborated with FADDL, ARS, and CSU to conduct FMD susceptibility and transmission studies in bison, elk, mule deer, and pronghorn. These studies were done at PIADC.

### **International Work**

**Poland:** In 1998, WiLDIT personnel, at the request of the National Park Service visited Bialowieza National Park in Poland to consult with Polish animal health authorities on a disease condition in European bison in the Park.

KITA J., K. ANUSZ, M. ZALESKA, E. MALICKA, W. BIELECKI, B. OSINSKA, B. KOWALSKI, Z. KRASINSKI, A. DEMIASZKIEWICZ, J. RHYAN, M. KOLIPINSKI. 2003. Relationships among ecology, demography and diseases of European bison (*Bison bonasus*). Polish Journal of Veterinary Sciences 6: 261-266.

**Israel:** In 2007, WiLDIT personnel and APHIS/WS personnel were invited to Israel to observe FMD in Israeli wildlife and domestic animals and to field trial infrared imaging as a surveillance tool in a disease outbreak.

**Workshop on Catastrophic Disease in Wildlife:** In partnership with CSU/APHI, WiLDIT hosted "A Workshop on the Science of Surveillance, Control, and Eradication of Catastrophic Diseases in Wildlife." This workshop was held in August 2007 and involved 30 international wildlife disease experts. A report on the workshop was produced. A final product of the workshop which is in preparation will be a handbook designed for managers on the subject.

### **Students**

WiLDIT personnel are adjunct staff members at CSU. In conjunction with CSU/APHI, WiLDIT assists in the education of numerous CSU students by providing project work for special-studies students, and graduate students. Additionally, WiLDIT offers externships for veterinary students and usually provides externships for 1 or 2 students annually. In the past, these have been from Washington, Ohio, Colorado, Virginia, and Brazil

**APHI/APHIS Wildlife Research Facility  
(Hockaday/Swanson Wildlife Research Facility\*)**

The **APHI /APHIS Wildlife Research Facility** occupies approximately 6.5 acres and is located on the Colorado State University Foothills Campus adjacent to the National Wildlife Research Center in Fort Collins, Colorado. The facility consists of multiple large paddocks with 8-foot high walls, while the entire perimeter of the site is surrounded by a 10-foot high fence. This facility contains handling equipment for deer, bison, elk, bighorn sheep, and other ungulate species. Security is provided by USDA/APHIS, National Wildlife Research Center and CSU security personnel. The animals and facility are maintained by a full time animal care staff and an attending veterinarian. All projects conducted at the facility are approved by the Colorado State University Animal Care and Use Committee.

\*Constructed, equipped, and funded primarily with end-of-year money and excessed property.

**List of Projects**

(Project summaries in Appendix A)

- 1. Biosafety of RB51 in adult bison bulls.** Cooperative project with USDA/ARS/NADC.
- 2. Lesions and tissue colonization sites of *B. abortus* in aborted bison fetuses and adult female bison from YNP.** Cooperative study with ARS/NADC, MTFWP, MTDOL, and DOI.
- 3. Experimental infection of cattle with marine mammal isolate of *Brucella*** Cooperative study with Washington DNR and USDA/ARS/NADC.
- 4. Brucellosis epidemiology and pathogenesis:** Cooperative study with ARS/NADC, MTFWP, DOI/BRD, and DOI/NPS.
- 5. RB51 dose-response study in bison.** Cooperative project with USDA/ARS/NADC.
- 6. Safety of RB51 in nontarget species: Groundsquirrels, voles, ravens, deer mice, pronghorn, and black bears.** Cooperative projects with ARS/NADC, APHIS/WS, and CODOW.
- 7. RB51 persistence in the GYA.,** Cooperative project with ARS/NADC, MTFWP DOI, and CEAH.
- 8. Fetus disappearance in the GYA study** Cooperative project with MTFWP.

- 9. Contraception in bison (3 projects).** Cooperative studies with CSU and APHIS/WS.
- 10. Contraception in elk (2 projects).** Cooperative projects with WYG&F and NPS.
- 11. Sustained release rifampin as therapy for brucellosis in bison.** Cooperative projects with APHIS/WS and CSU/APHI.
- 12. *Brucella ovis* in Bighorn Sheep.** Cooperative study with CSU/APHI.
- 13. Risk of *Brucella* transmission to bison and cattle posed by bison or elk abortion events in nature.**
- 14. Bison quarantine feasibility study.** Cooperative project with Montana Fish, Wildlife and Parks, Montana Dept of Livestock and CSU/APHI.
- 15. Recombinant RB51 *Brucella* vaccine in elk 1.** Cooperative study with ARS/NADC and CSU/APHI.
- 16. Recombinant RB51 *Brucella* vaccine in elk 2 Oral.** Cooperative study with ARS/NADC and CSU/APHI.
- 17. Serologic differentiation of infection with *B. abortus* from *Yersinia enterocolitica* O:9 in elk.** Cooperative project with CSU/APHI, LSU, NVSL, NADC and CAFIA.
- 18. CWD susceptibility of fallow deer.** Cooperative study with ARS/NADC and Colorado DOW.
- 19. CWD vaccine studies (One mouse study and one deer study).** Cooperative studies with APHIS/WS, Colorado Division of Wildlife, and ARS.
- 20. Oral TB vaccines in white-tailed deer.** Cooperative project with CSU/APHI and ARS/NADC.
- 21. Development of low risk sheep/goats for weeds.** Cooperative project with CSU/APHI and ARS. Goats from this study are now used for weed control around quarantined bison at Fort Collins.
- 22. FMD in North American bison.** Cooperative study with FADDL and ARS.
- 23. FMD in elk.** Cooperative study with FADDL, ARS, and CSU/APHI.
- 24. FMD in pronghorn.** Cooperative study with FADDL, ARS, and CSU/APHI.



- 25. FMD in mule deer.** Cooperative study with FADDL, ARS, and CSU/APHI.
- 26. Stress response in captive-raised and wild-caught bighorn sheep.**  
Cooperative project with CSU/APHI.
- 27. Infrared imaging for preclinical detection of FMD in mule deer.**  
Cooperative study with FADDL, ARS, and CSU/APHI.
- 28. Infrared imaging for remote reading of TB skin tests in elk.** Cooperative project with APHIS/WS and CSU/APHI.
- 29. Evaluation of positive molecular vaccine markers expressed in *Mycobacterium bovis* BCG in a ruminant model: Pilot Project.** Cooperative study with CSU/APHI, USDA/ARS, Albert Einstein College of Medicine, and Michigan State University.
- 30. Evaluation of two interferon gamma assays for diagnosis of bovine tuberculosis (Cervigam and Bovigam) in captive cervids.** Cooperative study with CSU/APHI, USDA/ARS, and Prionics.

### **Future Work:**

#### **GYA Brucellosis**

WiLDIT is committed to developing and implementing strategies to eradicate brucellosis from bison and elk in the GYA. Future work will determine on large samples sizes the duration of sterility produced by a single injection of GonaCon™, the immunocontraceptive vaccine. Future work will also produce a safe sustained-release antibiotic for decreasing *Brucella* infection in bison, and will explore the use of therapeutic vaccination in bison. These will conceivably be treatment modalities in a nonlethal strategy to eradicate brucellosis from bison. Developmental work in elk brucellosis will include further work in contraception, continued work on oral vaccination, and an "ecology of disease" study to produce a model for use in brucellosis elimination from elk. WiLDIT also will be involved in initiating and collaborating with CEAH and others on modeling efforts and cost-benefit analyses of brucellosis eradication.

#### **TB in Cervids**

WiLDIT has, in cooperation with CSU/APHI, obtained a grant to develop an immunologic marker for use in wildlife vaccines. Additionally, WiLDIT, in cooperation with APHIS/WS is developing baits for use in wildlife vaccines and is working toward a field trial in Michigan using the BCG formulation used in the previous white-tailed deer study. Current work is examining and comparing the use of Bovigam™ and Cervigam™, two tests for the analysis of gamma interferon, in elk.

**CWD**

In cooperation with APHIS/WS and CSU/APHI, WiLDIT has planned future work on the effect GnRH vaccine has on the development of CWD in mule deer. This is based on observations of GnRH use in elk that were exposed to CWD.

**Feral Swine**

WiLDIT, in cooperation with APHIS/WS and CSU/APHI, is developing an approach to dealing with diseases in feral swine. This approach utilizes temporary feeding sites, infrared surveillance for diseases, and pig specific feeders for poisoning, contracepting, or vaccinating feral swine. This approach might be utilized for several diseases including brucellosis, CSF, FMD, and influenza.

**Wildlife Disease Surveillance**

In cooperation with APHIS/WS, WiLDIT is involved in the development and validation of infrared surveillance for the screening of animals for certain diseases. These diseases include vesicular diseases in multiple species and febrile diseases in swine. The surveillance can be remotely conducted from fixed sites, and from manned and unmanned aircraft.

**FMD**

In cooperation with ARS, FADDL, and CSU/APHI, WiLDIT plans future studies to evaluate FMD vaccines for use in wildlife, specifically bison, white-tailed deer, and feral swine. The end-goal is the development of oral vaccines and delivery systems for use in wildlife for FMD and other diseases (CSF, ASF, etc.). These vaccines would be similar in application to the oral wildlife rabies vaccine currently in use.

# Yellowstone Bison Quarantine Study & Control

## Non-lethal

- Bison were gathered from Yellowstone National Park and relocated to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.
- The United States Department of Agriculture, Animal and Plant Health Inspection Service, and Veterinary Services are striving to eliminate the brucellosis infection in bison using a non-lethal method.



History of  
Bison migrated from Asia to North America about 10,000 years ago. The American bison once numbered in the millions, but they were hunted to near extinction. The American Bison Society was formed in 1906 to create wildlife preserves for the bison. There were about 500,000 bison in North America in 1906, but they were raised privately for meat and have since been reintroduced. It is important that conservation efforts serve the original trust.



# Contraception Project to Eliminate Brucellosis in a Natural Way

## History of Bison

North America nearly 200,000 years ago. Estimated to have numbered more than 60 million, until the late 1800s. In 1905 the U.S. government decided and persuaded congress to create a national bison herd. Today there are approximately 4,000 bison. Approximately 90% of bison are descended from bison that have been crossed with cattle lines. It is important that efforts continue to be made to preserve pure bison bloodlines.



- The Yellowstone Bison Quarantine Feasibility Study & Contraception Project is a seven year study. The bison were originally collected in June of 2011.
- The end of the study will be approximately October of 2017.



- The main objective of this study is to evaluate the efficacy of GonaCon as an immunocontraceptive vaccine in *B. abortus* infected female bison.
- As well as to evaluate the shedding of temporarily infertile female *B. abortus*-infected bison.



- The GonaCon Immune is being tested on its ability to prevent the shedding of brucella bacteria.
- GnRH is a naturally occurring hormone that signals the production of eggs.
- GonaCon interferes with the release of GnRH to signal hormones responsible for fertility.
- The use of GonaCon to prevent the shedding of *B. abortus* infected female bison could prevent potential abortions of the cycle. Transmission of the bacteria could be eliminated or possibly eliminated.

Poster by Zaneta McGhee & Victoria Martin

Investigators



contraceptive Vaccine is  
ability to prevent the shed

occurring hormone that  
n of sex hormones.

with the ability of GnRH  
resulting in temporary in-

to prevent pregnancy in  
male bison will eliminate  
affected bison, breaking  
n can then be decreased  
d.



- Bovine Brucellosis is a zoonotic bacterial disease caused by *Brucella abortus* (*B. abortus*) and can be passed amongst animals such as cattle, bison, and elk.
- Females will often abort pregnancies or give birth to weak calves, all resulting in shedding of the organism, which is the most common route of transmission.
- In humans, brucellosis is often called undulant fever because it persists for several weeks or months and may become progressively worse if left untreated.

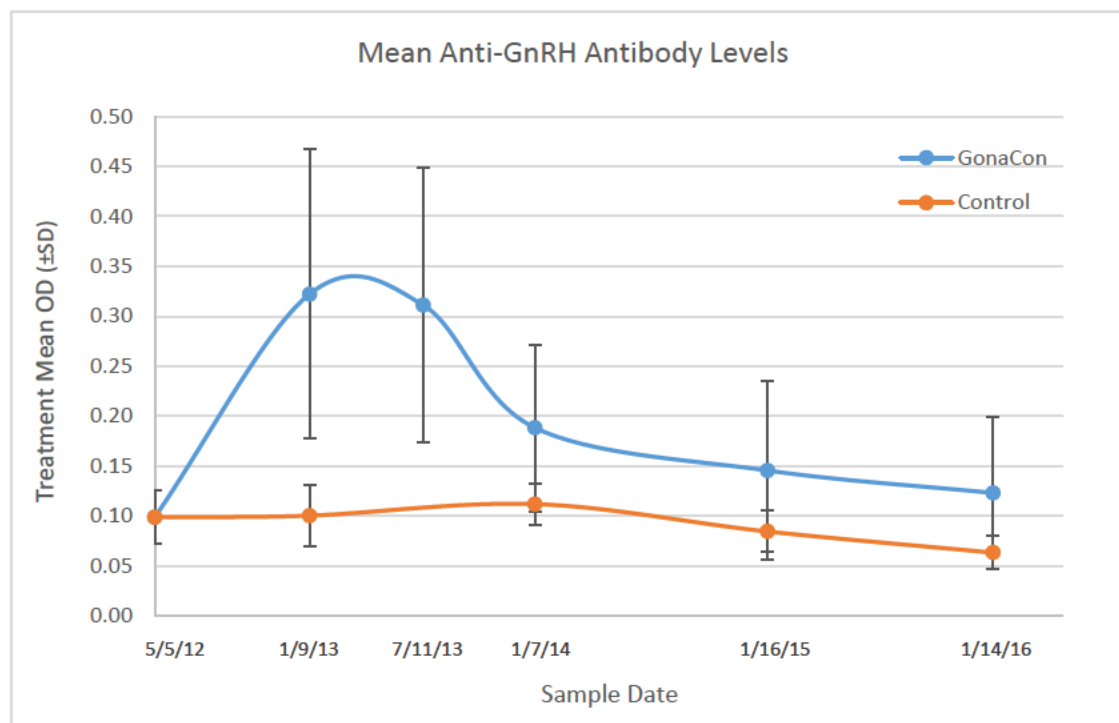


: Jack Rhyan, Pauline Nol, Matt McCollum, Rebecca Frey, Ryan Clarke, Luke Wagner, Lowell Miller and Jeff Kemp



**QA-1858: Evaluation of GonaCon, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**

**Figure 1: Bison mean anti-GnRH antibody levels by treatment (GonaCon versus control) 2012-2016**



**Table 1: Proportion of bison positive for anti-GnRH antibodies at each sampling time point (GonaCon versus control) 2012-2016**

	<u>GonaCon</u>		<u>Control</u>	
	n positive	% positive	n positive	% positive
5/5/2012	0/15	0%	0/13	0%
1/9/2013	12/15	80%	0/13	0%
7/11/2013	12/15	80%	n/a	n/a
1/7/2014	6/15	40%	0/13	0%
1/16/2015	2/14	14%	0/12	0%
1/14/2016	1/14	7%	0/12	0%

- Mean anti-GnRH antibody levels in GonaCon-vaccinated bison were significantly higher ( $\alpha=0.05$ ) at all sampling dates post-vaccination compared to control animals.
- Mean anti-GnRH antibody levels in GonaCon-vaccinated bison were significantly higher ( $\alpha=0.05$ ) at all sampling dates through 2015 compared to pre-vaccination background.

**Table 2:** Anti-GnRH antibody levels for GonaCon-treated bison; green-highlighted cells are greater than or equal to the positive/negative threshold and are therefore classified as positive for anti-GnRH antibodies.

Animal ID	Trt	Mean OD					
		5/5/2012	1/9/2013	7/11/2013	1/7/2014	1/16/2015	1/14/2016
R01	GC	0.117537	0.393458	0.464629	0.239448	0.149895	0.125247
R02	GC	0.127681	0.128227	0.236657	0.104571	0.072296	0.067039
R04	GC	0.064362	0.370542	0.440902	0.176683	0.149884	0.088046
R05	GC	0.101377	0.514980	0.483968	0.264644	0.177668	0.155059
R11	GC	0.146000	0.345574	0.442663	0.210741	0.125796	0.118187
R14	GC	0.094468	0.323310	0.347799	0.168831	0.189484	0.088942
R19	GC	0.068535	0.328592	0.266099	0.147381	0.085057	0.055830
R20	GC	0.052129	0.072882	0.206661	0.126105	0.099440	0.094822
R23	GC	0.089776	0.289521	0.281085	0.158610	n/a	n/a
R24	GC	0.125504	0.093721	0.176772	0.099320	0.062943	0.077142
R26	GC	0.067019	0.220960	0.176719	0.096015	0.079477	0.123372
R27	GC	0.075154	0.457168	0.299756	0.160664	0.104850	0.095936
R28	GC	0.063716	0.290777	0.001461	0.191367	0.143157	0.095733
R29	GC	0.082172	0.527690	0.432735	0.415462	0.419544	0.360588
R31	GC	0.152563	0.474654	0.408516	0.264790	0.179520	0.177879
Mean		0.098915	0.322137	0.311095	0.188309	0.145644	0.123130
StDev		0.027084	0.144703	0.137510	0.083477	0.089279	0.075912

Positive/Negative Threshold = pre-vaccination samples mean OD + 3StDev

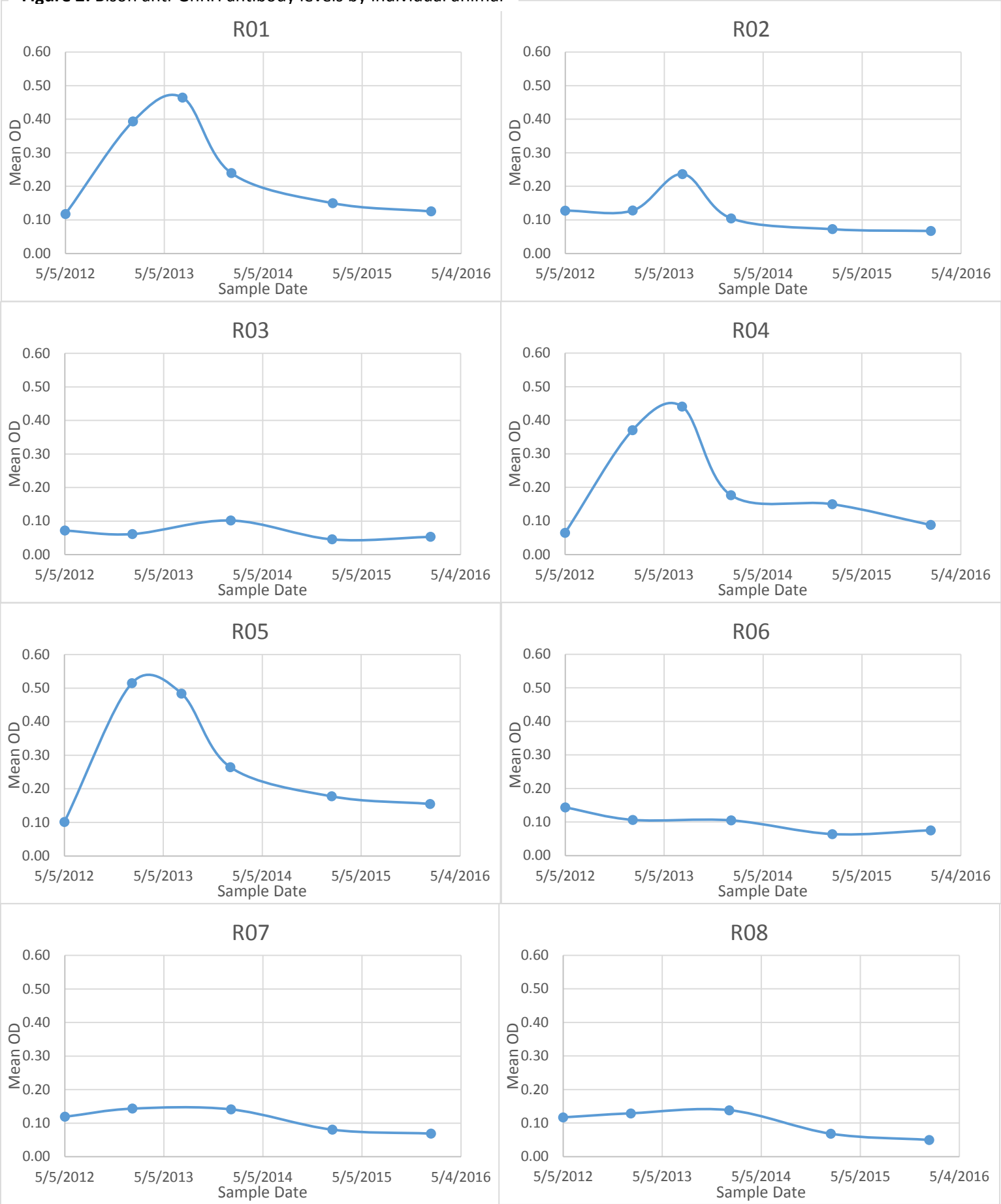
> or = 0.180166 POSITIVE

**Table 3:** Anti-GnRH antibody levels for control bison (*not* vaccinated with GonaCon)

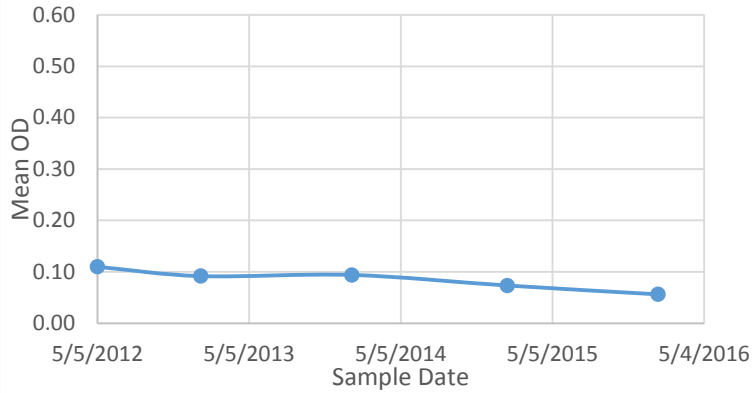
Animal ID	Trt	Mean OD					
		5/5/2012	1/9/2013	7/11/2013	1/7/2014	1/16/2015	1/14/2016
R03	ctrl	0.071843	0.061363	n/a	0.101848	0.045558	0.053084
R06	ctrl	0.143653	0.106539	n/a	0.105081	0.064216	0.075531
R07	ctrl	0.118956	0.143362	n/a	0.141152	0.080688	0.069023
R08	ctrl	0.117083	0.129192	n/a	0.138540	0.068364	0.049858
R09	ctrl	0.109698	0.091654	n/a	0.093941	0.073341	0.056111
R13	ctrl	0.107245	0.088282	n/a	0.122621	0.098129	0.055027
R15	ctrl	0.101152	0.118689	n/a	0.093021	n/a	n/a
R16	ctrl	0.132283	0.136006	n/a	0.114401	0.087604	0.073000
R17	ctrl	0.090685	0.108359	n/a	0.092384	0.073264	0.063970
R18	ctrl	0.082545	0.100041	n/a	0.086549	0.076670	0.067109
R21	ctrl	0.087038	0.070065	n/a	0.120418	0.053118	0.056085
R22	ctrl	0.064621	0.038263	n/a	0.100067	0.077518	0.038244
R25	ctrl	0.098533	0.114424	n/a	0.146134	0.125687	0.103691
Mean		0.098915	0.100480	n/a	0.112012	0.084570	0.063394
StDev		0.027084	0.030418	n/a	0.020250	0.020819	0.016536



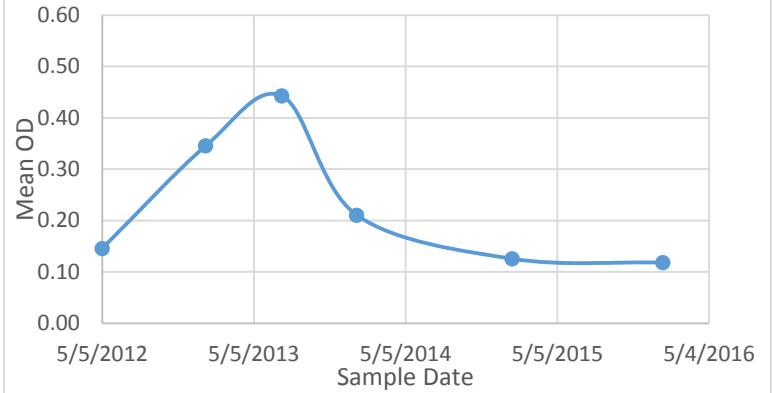
**Figure 2:** Bison anti-GnRH antibody levels by individual animal



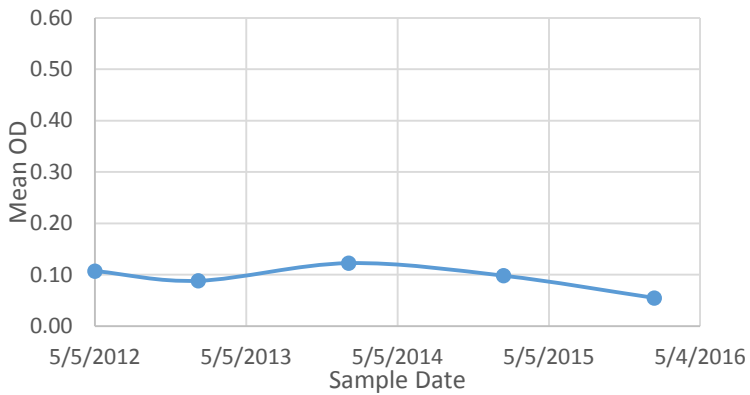
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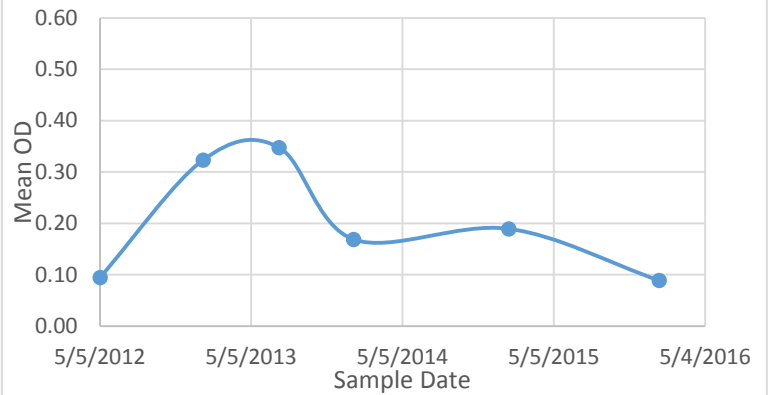
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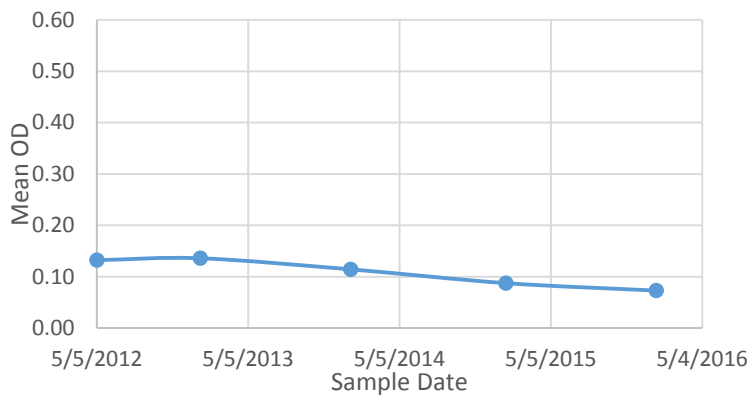
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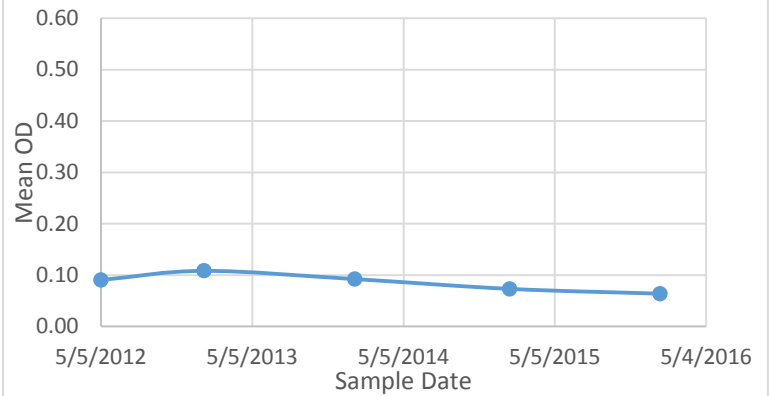
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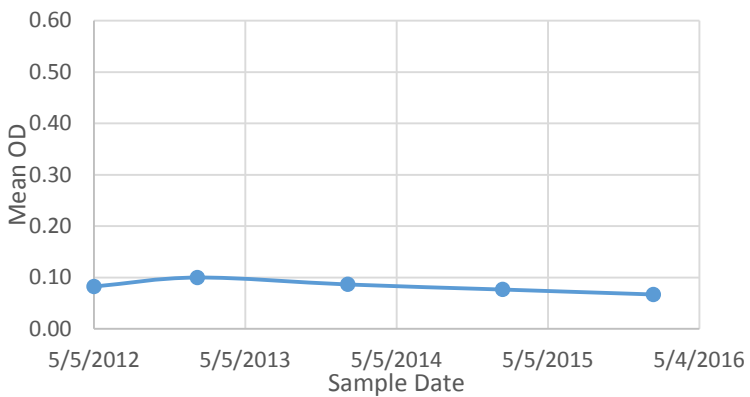
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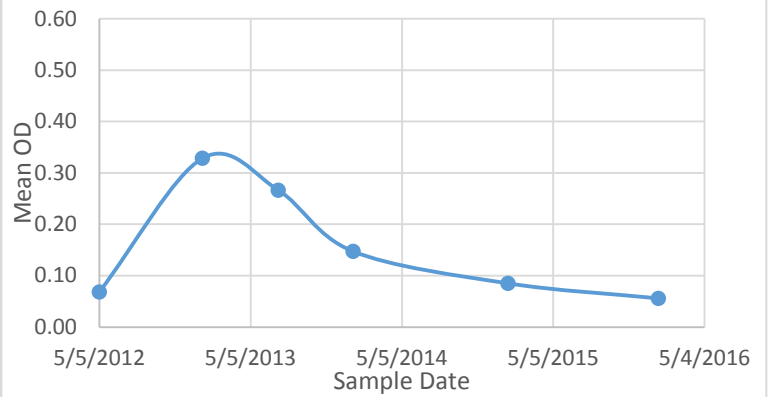
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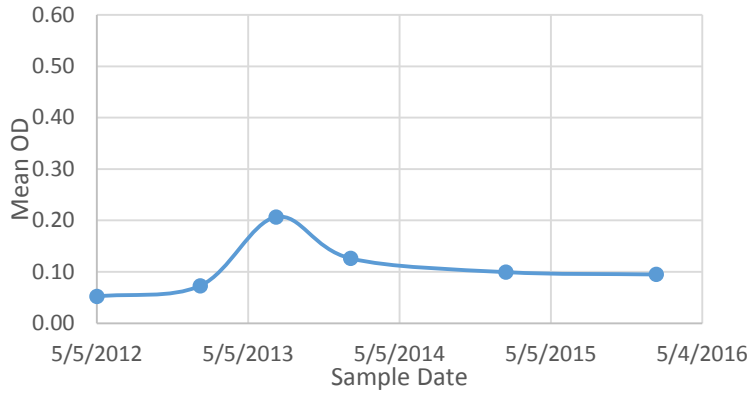
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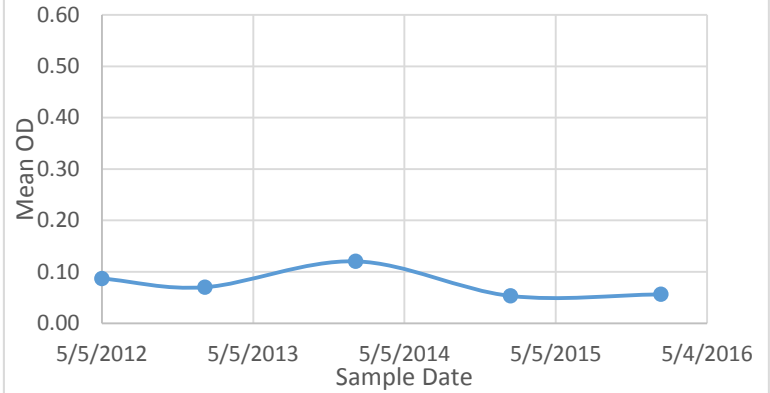
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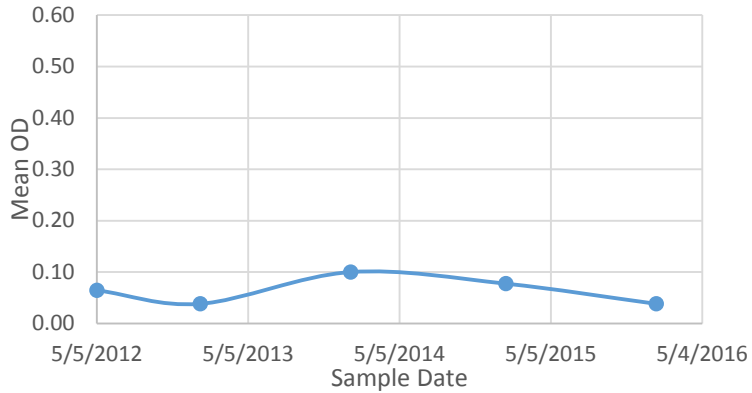
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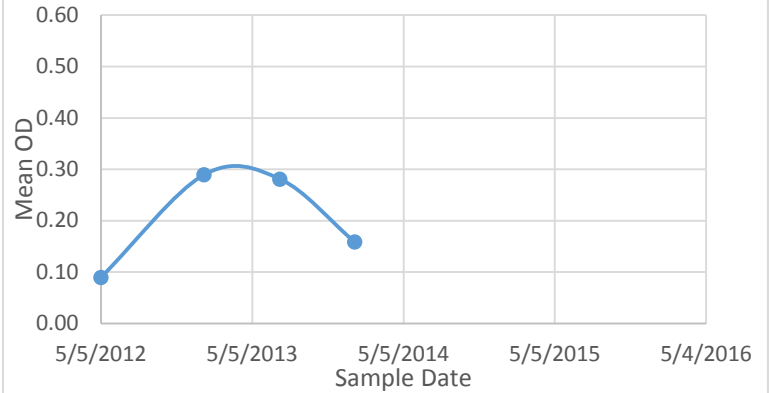
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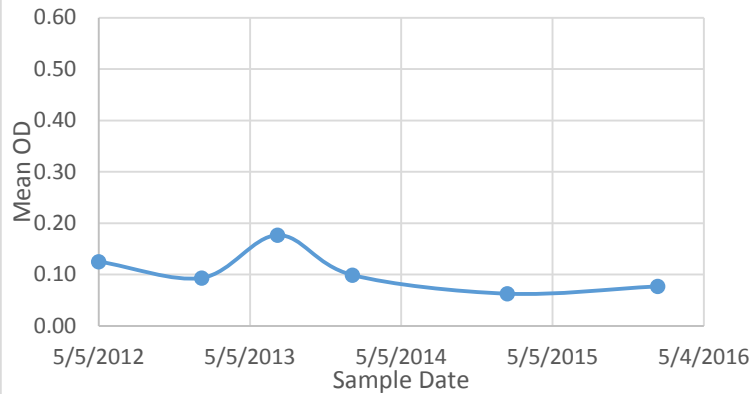
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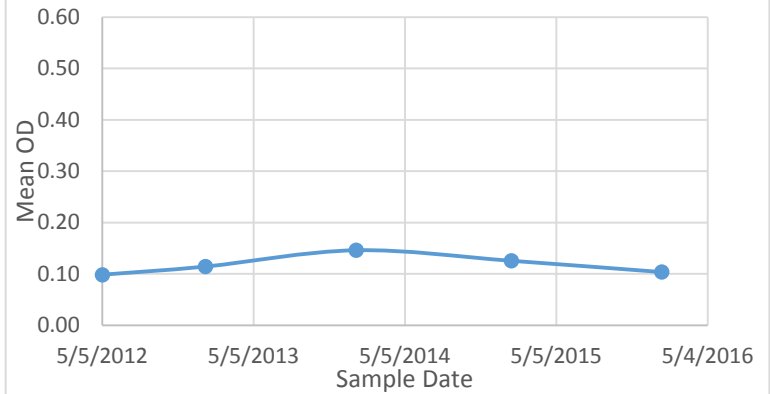
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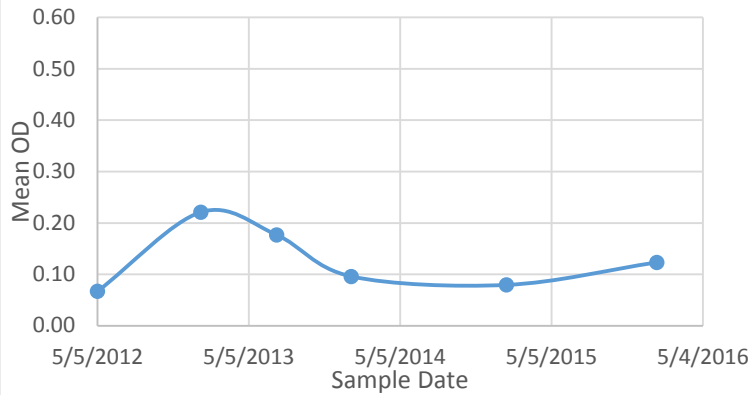
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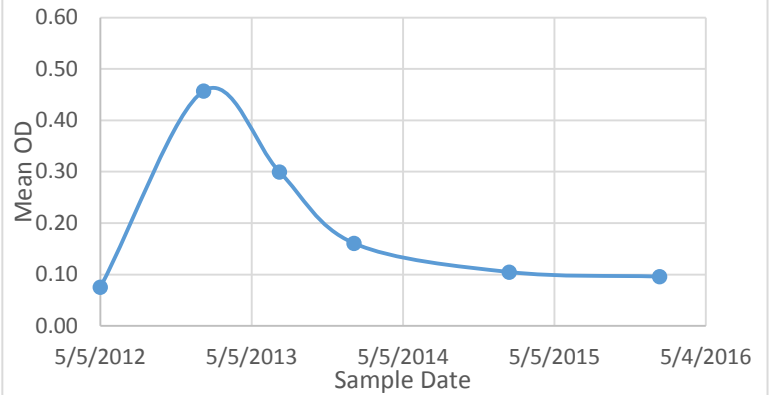
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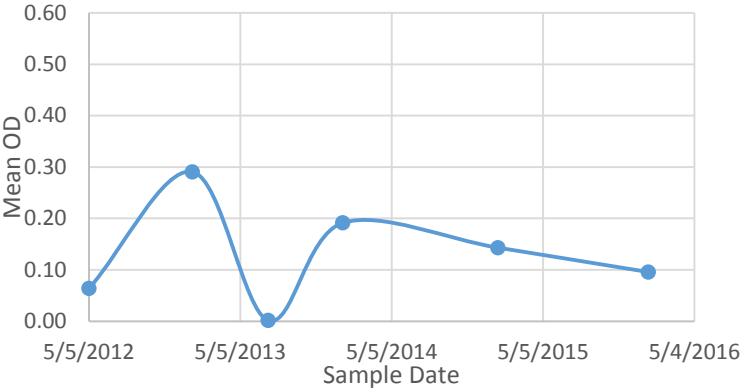
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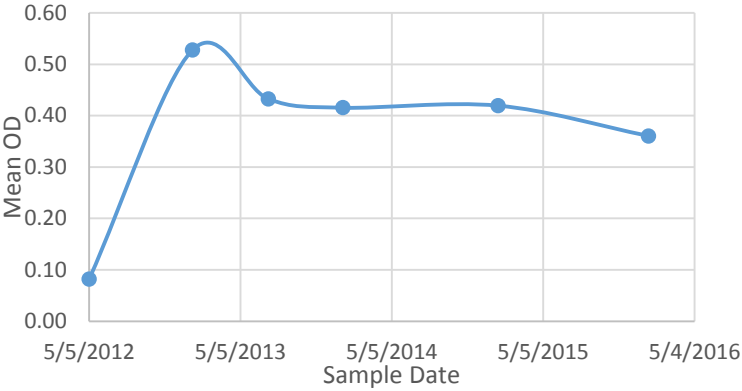
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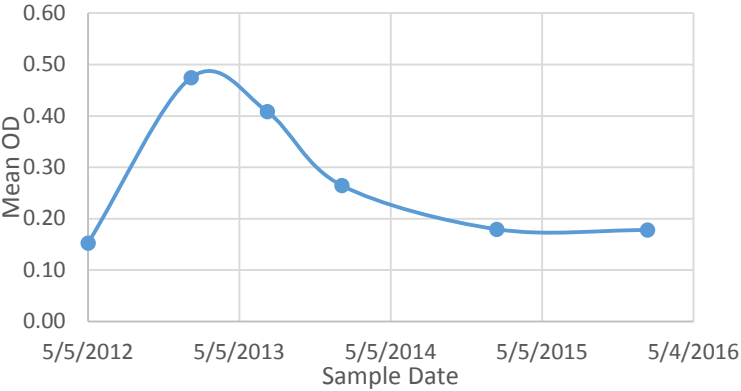
R28



R29



R31



## 1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services  
National Wildlife Research Center

### PROTOCOL COVER PAGE

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

<b>1</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, <b>and</b> there is generally no commitment of NWRC resources other than personnel time, <b>and</b> activities are not regulated research activities.</p> <p><u>Complete &amp; 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"> <li>• Writing or collaborating on review papers and synthesis reports</li> <li>• Student committee participation</li> <li>• Analyzing or writing up data collected under operational or other contexts</li> </ul>
<b>2</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, <b>but</b> the activity involves regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; 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"> <li>• Training programs requiring the use of animals</li> <li>• Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)</li> <li>• Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)</li> </ul>
<b>3</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>but</b> the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• Collaborating on study design, data analysis, or economic analysis.</li> <li>• Minor participation on a regulated study at the collaborating host institution</li> <li>• A study that does not include animal use, etc.</li> </ul>
<b>4</b> <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>and</b> the study includes regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• A typical NWRC led study</li> <li>• Major NWRC staff participation in regulated activity</li> <li>• Study takes place on NWRC facilities</li> </ul>

\* 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".



NO	YES	Item
<b>Animal Use</b>		
<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 <b>NWRC Animal Use Appendix</b> <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach <b>IACUC protocol &amp; approval</b> <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.
<b>Microbiological/Biohazardous Materials</b>		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach <b>Microbiological/Biohazardous Materials Use Appendix</b> .
<b>Permits</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. <u>          National Park Service                </u> <u>YELL-2011-SCI-5892                </u> May 10, 2011 <u>                </u> Permit(s) description <u>                                                                </u> Number <u>                </u> Date <u>                </u>
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>		
<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 <b>NEPA &amp; ESA Appendix</b> .
<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 <b>NEPA &amp; ESA Appendix</b> . 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.
<b>Regulatory Standard and Test Guidelines</b>		
<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: <u>                                                                                        </u>
<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:
<b>Test, Control and Reference Material/Devices</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the <b>Test, Control and Reference Material/Devices Formulation and Use Appendix</b> . Please indicate if otherwise described in the protocol.
<b>Historical Resources</b>		
<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.
<b>Material Transfer Agreement</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate <b>Material Transfer Agreement</b> . Material Transfer agreements will be developed prior to material transfer
<b>Analytical Chemistry</b>		
<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 <sup>th</sup> and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

### 3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

### 4. Schedule

Proposed Experimental Start Date: April 15, 2012  
 Proposed Experimental Termination Date: October 1, 2017  
 Proposed Study Completion/Archive Date: October 1, 2019

### 5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily



through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

## 6. Related Protocols

- |      |                                                                                                                                                                             |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer ( <i>Odocoileus virginianus</i> ): Pivotal target animal safety study                                             |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission                                                                                                 |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland                                                    |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey                                                  |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland              |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer ( <i>Dama dama</i> ) at Point Reyes National Seashore, California            |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk ( <i>Cervus elaphus</i> ) at Rocky Mountain National Park, Colorado                  |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses ( <i>Equus caballus</i> ) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer                                                                                                                                 |

## 7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

## 8. Objective/Hypotheses

### Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

### Null Hypotheses:

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

## 9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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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 <sup>st</sup> replicate			
2012	Collect bison for 1 <sup>st</sup> and 2 <sup>nd</sup> replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 <sup>nd</sup> replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 <sup>nd</sup> replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

## 11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

## 12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a. Animal handling and sample collection records
  - b. Necropsy records
  - c. Results of serologic, histopathologic, and cultural analysis
  - d. Animal calving observation records
  - e. Pregnancy assessment records
- D. Final Report

## 13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400
E. Animal Care Cost	\$0	\$0	\$0					
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900

## 14. Human Health and Safety

HS004-00	Personal protective equipment
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## 15. Staff Qualifications

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All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

## 16. Archiving

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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

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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

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Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer

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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

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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

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## Animal Use Appendix

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**A). Animal Information:**

Species, subspecies (if applicable): Bison (*Bison bison*)  
Breed, strain and substrain (if applicable): NA  
Total Number and Sex: 96 females, 8 males  
Body weight range: 400-1000 kg  
Age: 2 year to adult

**B1) Rationale for involving animals:**

This study must be conducted in bison which are the target species of management. These data cannot be collected in an in vitro setting.

**B2) Rationale for numbers to be used:** If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

**B3) Rationale for appropriateness of the species to be used:** Bison are the target species.

**C) Source:** Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

**D) Method of identification of animals:** Animals will be ear tagged and microchipped for identification.

**E) Trapping/Collecting:** Animals will be captured by National Park Service personnel as part of the ongoing Interagency Bison Management Plan according to agency protocol.

**F) Transport:** Animals will be loaded on to stock trailers and transported to the Corwin Springs facility. The Corwin Springs facility is within 2 miles of the NPS capture facility.

**G) Handling/restraint:** Handling facilities consist of alleyways leading to a standard cattle manual squeeze chute that has been modified to accommodate bison. In the event that animals must be chemically restrained they will be darted with a combination of opioid narcotics and alpha-2 adrenergics.

Drugs: A3080- 0.01-0.015 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Carfentanil-0.005-0.01 mg/kg, IM dart  
Xylazine- 0.07 mg/kg, IM dart  
  
Butorphenol- 0.03-0.06 mg/kg, IM dart  
Medetomidine- 0.01-0.02 mg/kg  
Azaperone- 0.02 mg/kg

Reversal for narcotics:

Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given



Tolazoline-300 mg as needed IM

Reversal for BAM:

Atipamezole 0.0375-0.03 mg/kg IM

Naltrexone 0.05-0.125mg/kg IM

Tolazoline 1 mg/kg IM

- I) Housing/maintenance: The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. Animals are to be maintained on pasture when available, hay ad libitum in winter, and fresh water at all times.

**J) Dietary contaminant exposure NA**

**K) Disposition of animals:** It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring that remain or become seropositive for *B. abortus* after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation.

**L) Animal pain or distress**

L1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: \_\_\_\_ Patrick Ryan Clarke \_\_\_\_\_

Date of Consultation: \_\_\_\_ 13 May 2011 \_\_\_\_\_

L2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☒ No

☐ Yes If yes, continue with the following items.

a) Alternative procedures:

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

c) Surgery:

**M) Euthanasia**

It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is mortally injured during routine handling, euthanasia will be performed by trained personnel using a captive bolt, a bullet from a high powered rifle, or appropriate chemical euthanasia solutions. Animals will be chemically immobilized prior to euthanasia when appropriate. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

**N. IACUC Approval**

Date of IACUC Approval Letter: \_\_ACUC Protocol approved 5/17/2011\_ See attached\_\_

Bison Quarantine Facility Institutional Animal Care and Use Committee

**O. Staff Qualifications**

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. See section 15 in protocol.

## NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

**Effects on T&E species and eagles:**

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

**Consultations:**

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyan has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

## Test, Control and Reference Material/Devices Formulation and Use Appendix

### A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
  - a) Concentration and purity: 1000ug/ml purity:na
  - b) Source: National Wildlife Research Center
  - c) Batch number: to be determined

### B. Describe any control or reference materials/devices

No control or reference materials will be used

### C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

### D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

### G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

ACP Consultation:

## 1.1 United States Department of Agriculture

Animal and Plant Health Inspection Service/Wildlife Services  
National Wildlife Research Center

### PROTOCOL COVER PAGE

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

<b>1</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, <b>and</b> there is generally no commitment of NWRC resources other than personnel time, <b>and</b> activities are not regulated research activities.</p> <p><u>Complete &amp; 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"> <li>• Writing or collaborating on review papers and synthesis reports</li> <li>• Student committee participation</li> <li>• Analyzing or writing up data collected under operational or other contexts</li> </ul>
<b>2</b> <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, <b>but</b> the activity involves regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; 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"> <li>• Training programs requiring the use of animals</li> <li>• Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)</li> <li>• Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)</li> </ul>
<b>3</b> <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>but</b> the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• Collaborating on study design, data analysis, or economic analysis.</li> <li>• Minor participation on a regulated study at the collaborating host institution</li> <li>• A study that does not include animal use, etc.</li> </ul>
<b>4</b> <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, <b>and</b> the study involves NWRC facilities and staff, <b>and</b> the study includes regulated research activities*.</p> <p><u>Complete &amp; 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) &amp; approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> <li>• A typical NWRC led study</li> <li>• Major NWRC staff participation in regulated activity</li> <li>• Study takes place on NWRC facilities</li> </ul>

\* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

## PART ONE: SIGNATURE PAGE

Study Director: Jade C. Myers Date: 2/17/12

Position (check one):

☐ Biologist/Chemist/Technician  
Supervisor signature required:

\_\_\_\_\_ Date \_\_\_\_\_ ☐ Res. Scientist ☐ Proj. Leader

☒ Research Scientist☐ Project Leader

☒ Visiting Scientist: NWRC Representative/Contact: LOWEN MILLER

☐ Student: NWRC Representative/Contact: \_\_\_\_\_

Concur: \_\_\_\_\_  
NWRC Research Project Leader Jacob Ryan Date 2/17/12

Review and Processing: L. Heimer Date 2/21/12

Concur:  
NWRC Assistant Director Mark E. Robin Date 2/22/12

Approved: \_\_\_\_\_ Date 2/22/12

Note: Additional approvals are located in the attached appendices.

## PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
<b>Animal Use</b>		
<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 <b>NWRC Animal Use Appendix</b> <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach <b>IACUC protocol &amp; approval</b> <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.
<b>Microbiological/Biohazardous Materials</b>		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach <b>Microbiological/Biohazardous Materials Use Appendix</b> .
<b>Permits</b>		
<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 _____
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>		
<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 <b>NEPA &amp; ESA Appendix</b> .
<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 <b>NEPA &amp; ESA Appendix</b> . 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.
<b>Regulatory Standard and Test Guidelines</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: <u>June 2, 2011</u>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i> <input type="checkbox"/> Other: _____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
<b>Test, Control and Reference Material/Devices</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the <b>Test, Control and Reference Material/Devices Formulation and Use Appendix</b> . Please indicate if otherwise described in the protocol.
<b>Historical Resources</b>		
<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.
<b>Material Transfer Agreement</b>		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate <b>Material Transfer Agreement</b> . Material Transfer agreements will be developed prior to material transfer
<b>Analytical Chemistry</b>		
<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 FOUR: FULL NWRC STUDY PROTOCOL

### 1. Key Personnel

Name	Organization	Role in Study
<b>Study Director</b>		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
<b>Other Investigators, Collaborators, Cooperators, and Consultants</b>		
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 <sup>th</sup> and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

### 3. Sponsor

Name	Address	Contract No.
USDA/APHIS VS Western Regional Office	2150 Centre Ave, Fort Collins, CO	NA
USDA/ APHIS NWRC	4101 W Laporte Ave, Fort Collins CO	NA

### 4. Schedule

Proposed Experimental Start Date: April 15, 2012  
 Proposed Experimental Termination Date: October 1, 2017  
 Proposed Study Completion/Archive Date: October 1, 2019

### 5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

## 6. Related Protocols

- |      |                                                                                                                                                                             |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1209 | GonaCon Immunocontraceptive Vaccine for White-tailed Deer ( <i>Odocoileus virginianus</i> ): Pivotal target animal safety study                                             |
| 1451 | GonaCon immunocontraceptive vaccine for use in cervids: EPA data submission                                                                                                 |
| 1112 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in Maryland                                                    |
| 1277 | Pivotal field study of GonaCon immunocontraceptive vaccine for use in the contraception of white-tailed deer in New Jersey                                                  |
| 1417 | Collection of ancillary data on GonaCon Immunocontraceptive vaccine use during autumn and winter for the contraception of female white-tailed deer in Maryland              |
| 1445 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of Fallow deer ( <i>Dama dama</i> ) at Point Reyes National Seashore, California            |
| 1523 | Field study of GonaCon immunocontraceptive vaccine for use in the contraception of elk ( <i>Cervus elaphus</i> ) at Rocky Mountain National Park, Colorado                  |
| 1657 | Field study of GonaCon Immunocontraceptive Vaccine for use in the contraception of feral horses ( <i>Equus caballus</i> ) at Theodore Roosevelt National Park, North Dakota |
| 1216 | Chemical sterilization of black-tailed deer                                                                                                                                 |

## 7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:

PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

## 8. Objective/Hypotheses

### Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

### Null Hypotheses:

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

## 9. Methods/Procedures

A total of 96 female bison (yearlings, two- and three-year-olds –approximately 24 seronegative and 72 seropositive and 4-8 seronegative bulls captured in late winter/spring 2011, 2012, 2013, and 2014 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute. Blood will be collected from the jugular vein or tail vein.

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations; no cattle are present within a mile of the facilities. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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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 <sup>st</sup> replicate			
2012	Collect bison for 1 <sup>st</sup> and 2 <sup>nd</sup> replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2 <sup>nd</sup> replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2 <sup>nd</sup> replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

## 10. Experimental Design and Statistical Analyses

In a study of naturally infected bison in Yellowstone National Park, 3/10 cows (30%) aborted their first calves after seroconversion (Rhyan et al., 2009) and the data suggested that recently seroconverted cows were at highest risk for shedding of *B. abortus*. Although we will be targeting younger seropositive animals in order to increase chances of recent seroconversion, we may have to collect older animals depending on circumstances and we will not have a good idea of when seroconversion occurred. We therefore estimate that at least 1 in 10 seropositive animals would abort or shed *Brucella* if allowed to breed.

If we expect an abortion rate of 5-10% in the vaccinated group and a 30% abortion rate in the non-vaccinated group, then, with 18 seropositive animals per pen we have an 82% power to detect a 23% change (30% to 7% abortions). Two replicates of the two pastures will be conducted.

## 11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA

AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

## 12. List of Records to be Maintained

- A. Protocol and Amendments
- B. Correspondence, telephone logs and related records
- C. Data records including:
  - a. Animal handling and sample collection records
  - b. Necropsy records
  - c. Results of serologic, histopathologic, and cultural analysis
  - d. Animal calving observation records
  - e. Pregnancy assessment records
- D. Final Report

## 13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	\$900	\$900	\$900	\$900	\$900	\$900	\$900	\$900
B. Facilities	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
C. Equipment	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
D. Supplies	\$400	\$400	\$400	\$400	\$400	\$400	\$400	\$400
E. Animal Care Cost	\$0	\$0	\$0					
F. Operating Costs	\$600	\$600	\$600	\$600	\$600	\$600	\$600	\$600
TOTAL	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900	\$1,900

## 14. Human Health and Safety

HS004-00	Personal protective equipment
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## 15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

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## 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

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## 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.

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## 18. References

Killian G., T. J. Kreeger J. C. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). J. Wildl. Dis. 45: 184-188.

Manthei, C. A., and R. W. Carter. 1950. Persistence of *Brucella abortus* infection in cattle. Am. J. Vet. Res. 11: 173-80

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. Immunocontraception of white-tailed deer

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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

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## Animal Use Appendix

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**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.

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## NEPA and ESA Appendix

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A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☒ This study does not qualify for a Categorical Exclusion. An EA is in development

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

Animals in this study were trapped by NPS and would otherwise have been taken to slaughter. Therefore, this study does not have impact on the bison population in the Greater Yellowstone Area.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

**Effects on T&E species and eagles:**

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

**Consultations:**

Did you consult with a state or federal agency specifically on this action.

☐ No

☒ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Jack Rhyon has had multiple conversations with the Montana State Veterinarian, Marty Zaluski. Dr. Zaluski is in favor of this study.

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

☐ No, permission not needed because:

☒ Yes Dennis Tilton, manager of the facility, is aware of and is in agreement with the execution of this study

## Test, Control and Reference Material/Devices Formulation and Use Appendix

### A. Describe the test material/devices

As appropriate, for each material provide the chemical, bait or device

- 1) name or code GonaCon™ Immunocontraceptive Vaccine
  - a) Concentration and purity: 1000ug/ml purity:na
  - b) Source: National Wildlife Research Center
  - c) Batch number: to be determined

### B. Describe any control or reference materials/devices

No control or reference materials will be used

### C. Carriers, mixtures and material preparation

Each 1.0 ml dose of GonaCon™ formulation contains the following ingredients:

GnRH/ Blue Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue)	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Distilled water	0.48 ml
AdjuVac™ adjuvant	
<i>Mycobacterium avium</i> (Mycopar™)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

### D. Route of administration

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the brisket. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1.5 ml on either side of the neck or hip. Landmark measurements will be taken prior to injection to identify the exact sites of injection and tattoo marking may also be utilized.

### F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

### G. Material verification

Manufacturing lot has already been verified by analytical chemistry and may be verified post-vaccination if deemed necessary. Method used is 167A Determination of GnRH in GonaCon immunocontraceptive vaccine

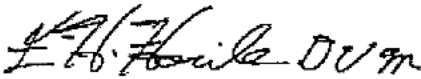
ACP Consultation:

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Friday, July 25, 2014 11:49 AM  
**To:** Fuentes, Antonio; Horak, Sarah; Knopp, Doug; Clarke,Patrick  
**Subject:** GonaCon study results

Case#8-45--15 Tube#10 81AJW3758 is classified as a reactor. The rest are negative.  
Case#8-37-15 Tubes #1,2&3 Red60,Red,58,Red57 are classified reactors the rest are negative.  
Case#8-40-15 Tube#6 YNP930797 is classified as a reactor , the rest are negative



Designated Brucellosis Epidemiologist

Regards and thanks Antonio Frank



This email is free from viruses and malware because avast! Antivirus protection is active.

## **Concerning potential slaughter of USDA research bison in Montana**

Decisions currently being made in Montana by a variety of agencies will significantly impact a valuable research program in the College of Veterinary Medicine and Biomedical Sciences concerning assisted reproductive technologies as a disease mitigation strategy for bison. A recent report indicates intent to slaughter a group of research bison that are currently being held in the Corwin Springs facility. These bison are offspring of bison from Yellowstone National Park that were designated for research by an agreement between USDA-APHIS and Yellowstone National Park and have been under USDA-APHIS management for the duration of their life. They have never been free roaming in Yellowstone National Park and their sero-status for *B. abortus* is known (3 *B. abortus* positive yearling bulls, 2 *B. abortus* positive yearling heifers, and 37 *B. abortus* negative cows, heifers, and calves). Immediately, 20 of the *B. abortus* negative bison may be sent to slaughter to make room for 25 bulls that USDA-APHIS will quarantine for Native American tribes.

As partners on this project, it is our understanding that it was the intent of our collaborators at USDA-APHIS-VS in Fort Collins and Montana to transfer these bison to the research facility on the Foothills Campus to continue our joint research. However, recent discussions on how to manage and potentially quarantine bison that migrate out of the park in the winter have resulted in the research bison being lumped into this discussion. The research bison are categorically different than the bison that are migrating out of the park, and we request that decisions made regarding the disposition of these animals be considered separately and with the full scope of their history in mind. Failure to do may result in healthy bison being sent to slaughter with significant impact on research. This request is not in opposition to or in conflict with requests for bison by tribes and in those cases, if an agreement is reached between the involved parties, then we look forward to supporting those efforts.

As these conversations have escalated there appears to be a strong possibility for the research bison to be sent to slaughter without considering other options; we have the space in our facility to accommodate these animals. Following are the two primary reasons that have been given for sending the bison to slaughter.

### **Argument 1: It would be unfair to move bison to the USDA-APHIS facility in Fort Collins and not give them to the tribes.**

Native American Tribes have requested that bison from Yellowstone National Park be moved to Fort Peck Reservation in Montana for the completion of a quarantine program. These potential “quarantine bison” are ones that were born in Yellowstone National Park, have lived their lives free-ranging in the greater Yellowstone area, but were captured north of the park during the annual winter migration. These bison will need to be quarantined according to the USDA’s Brucellosis Eradication Uniform Methods and Rules 2003 (APHIS 91-45-013).

Colorado State University (CSU) is not requesting these potential quarantine bison, nor has CSU ever been the recipient of quarantine bison. Likewise, CSU does not desire to become a quarantine facility for bison from Yellowstone National Park. CSU desires to continue valuable research in partnership with USDA-APHIS on assisted reproductive technologies that can be used in disease mitigation strategies for bison, which is currently directed under



a memorandum of understanding between CSU and USDA-APHIS-VS. As such, this request is for bison to be transferred from the APHIS research facility in Montana to the APHIS research facility on the CSU campus in Fort Collins, CO, not to be transferred to CSU directly.

All bison that have been made available to CSU researchers have been allocated for research to USDA-APHIS-VS in Fort Collins, Colorado and remained the property of USDA-APHIS for the duration of the research. Transfer of bison to USDA-APHIS has been achieved through an agreement between USDA-APHIS and Yellowstone National Park and approved by the Interagency Bison Management Plan, a cooperative, multi-agency effort that guides the management of bison and brucellosis in and around Yellowstone National Park ([www.ibmp.info](http://www.ibmp.info)). Objective 2.2 of the IBMP is to “minimize bison slaughter by employing alternative management techniques.” One of the acceptable dispositions of Yellowstone bison as decided by the IBMP has been to transfer bison that would have otherwise been slaughtered to USDA-APHIS to conduct research to further understand brucellosis in bison and develop potential strategies for dealing with the disease in the greater Yellowstone area. This is in accordance with the management action of objective 2.2 which is to “use slaughter only when necessary (e.g., disease suppression by selectively removing likely infectious bison); attempt to use other risk management tools first.” In this case, slaughter is not necessary. These bison are wanted for research in Colorado and there is space in the approved research facility operated by USDA-APHIS on the Foothills Campus of CSU.

CSU requests that the research bison described above be approved for transfer to the APHIS research facility in Fort Collins, CO. CSU and the USDA-APHIS research teams have jointly planned future research on the belief that members of the IBMP would continue to uphold the objectives of the management plan, including using slaughter as a last resort. Should the agencies involved decide to send the research bison to slaughter, it would be in direct conflict with objective 2.2 of the plan, as slaughter is clearly not necessary. It would also effectively stop all research on assisted reproduction with live bison as a disease mitigation strategy at CSU, a project which has wide public support in Northern Colorado and has resulted in the establishment of a new conservation herd (Laramie Foothills Bison Conservation Herd) which has been nationally and internationally recognized. All eligible research animals in this program are returned to the landscape either through the Laramie Foothills herd or other conservation or tribal herds. A summary of our efforts to share these genetics can be provided.

**Argument 2: It is too risky to move bison that have been potentially exposed to *B. abortus* because it is a select agent.**

Using this rationale for sending bison to slaughter rather than sending them to the research facility in Colorado is problematic for a variety of perspectives. Bison from USDA-APHIS research facilities in Montana have been sent to Colorado for 15+ years. This movement has been done legally and with the approval of the state veterinarians of Montana and Colorado. If APHIS decides to alter their policies to treat bison as a select agent threat this will be problematic for more than just transport to the research facility in Colorado. All slaughterhouses that agree to slaughter bison from Yellowstone will have to be approved to handle select agent tissues and all tissues will have to be managed according to the designated chain of command (according to USDA Select Agent program). In addition, the

idea that bison naturally infected with *B. abortus* should be handled as select agent individuals would be problematic for all hunters of bison and elk and anyone who handles animals potentially infected in the greater Yellowstone area.

In addition, in an email to Dr. Jack Rhyan in 2010 regarding how to handle animals potentially positive for brucellosis, Freeda Isaac DVM, Director of the National Center for Import Export with USDA-APHIS-VS, stated that Select Agent Program directors met and agreed that for “naturally affected animals, samples taken from those animals would not be considered select agent material and required to be handled as restricted material until the sample was confirmed to have select agent material.”

In addition it is in direct contrast to a recent proposed change to the list of select agents issued by USDA-APHIS, in which it is requested that *B. abortus* (and *B. suis*) be removed from the select agent list as it is believed that the “effect of exposure to these agents on animal health and on the production and marketability of animal products is minimized.” The eradication of these organisms from the domestic livestock industry and the extensive regulatory programs in place for the remaining affected Designated Surveillance Area of *B. abortus* is referenced as rationale for removing *B. abortus* from the select agent list (Federal Register, vol 81, no 11,p2763; January 19, 2016).

While the majority of the bison we are requesting are negative for brucellosis, it is imperative to our research program that we are still able to acquire brucellosis positive animals. Without this subset of animals, we have no way to test how effective the reproductive techniques are at removing the bacterium and the risk of spreading the disease. We have been waiting to publish our successful results until we confirm them with more *B. abortus* positive bison. If progress is to be made, research will need to be part of the solution. Sending bison being requested for research to slaughter impedes progress, particularly when there is a pathway for *B. abortus* negative bison to return to the landscape and build bison populations outside of the park after participating in research.

## **Conclusion**

Getting healthy Yellowstone bison back on the landscape, including onto tribal lands, has been a goal for the agencies involved in bison management in Montana. As a genetically valuable herd, Yellowstone bison are invaluable for building a healthier, sustainable bison population. The collaboration between CSU and USDA-APHIS has opened up a new avenue of research with direct impact on being able to do this while contributing scientific knowledge on bison reproduction, a critical component of herd health. The establishment of the Laramie Foothills Bison Conservation Herd is a further demonstration of both CSU and USDA-APHIS's commitment to conservation of this species through research and stewardship. We sincerely hope that those making decisions will consider these contributions when making decisions about bison that are currently in the USDA-APHIS research program.

## Study Protocol Renewal/Amendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

### 2. Action needed:

       Project Completed

       Project Never Initiated

  X   Project On-going/Active: renew as approved

       Project On-going/Active: renew with minor revisions

       Project Not Yet Initiated or is Inactive: renewal requested

Anticipated start date                     

### 3. Protocol Changes

In the upcoming year will you implement any changes to the animal component of the project that differ from those in the original (or subsequent) approval by the IACUC (e.g. changes to animal procedures, number of animals needed, or project objectives)?

Yes        No   X  

### 3. Animal Use and Procedure alternatives since the last IACUC approval

a. Have alternatives to the use of animals become available that could be substituted to achieve specific project aims? Yes        No   X  

b. Have alternatives that are potentially less painful or distressful to animals become available that could be used to achieve specific project aims? Yes        No   X  

If you answered yes to either question, please provide a description below or attach one.

N/A

Animal usage (please complete the following box):

Enter one species in each box and report vertically	Bison
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	104
2. Number of animals used during first IACUC approval year	40
3. Number of animals used during second IACUC approval year (enter 0 if in future)	40
4. Number of animals used during third approval year	42
5. Number of animals used during fourth approval year	68
6. Number of animals used during fifth approval year	96
7. Number of animals used during sixth approval year	93

Study Director

  
Jack C. Rhyan

Date Dec 8, 2016

Concur

IACUC Chair

\_\_\_\_\_  
P. R. Clarke

Date \_\_\_\_\_

\_\_\_\_\_  
Dan Tyers

Date \_\_\_\_\_

\_\_\_\_\_  
Dennis Tilton

Date \_\_\_\_\_

## ***NWRC STUDY RECORDS***

***(Title contains 'GnRH')***

***(March 2, 2011)***

<b><i>Study Director</i></b>	<b><i>QA Number</i></b>	<b><i>Title</i></b>
Campbell	1549	Chemical sterilization of captive male shoats with a GnRH vaccine
	1783	Oral vaccination of feral swine with a GnRH vaccine
Kemp	1601	Efficacy testing of new GnRH peptide lots, adjuvant formulation changes for GonaCon production, and a novel French Immunocontraceptive protein
	1696	Bioassay to evaluate the efficacy of a recombinant anti-GnRH protein
	1786	Oral delivery of Salmonella choleraesuis vaccine vector expressing a recombinant multimeric GnRH protein
Mathies	1233	Effect of a GnRH vaccine on male brown tree snake reproduction
Mauldin	1769	Porcine Tonsillar and Ileal uptake and transport of oral vaccines: GnRH, Mycobacterium avium, BCG, and RB-51
Miller	1287	Efficacy and comparison of vitrified injectable and vitrified oral GnRH immunocontraception of rabbits
Nash	911	Test of GonaCon (GnRH vaccination) treatment in an urban Berkeley, California population of California ground squirrels as a population management tool
Powers	933	Evaluation of Adjuvac emulsion as an alternative to Freund's complete and incomplete adjuvant as a carrier for GNRH-KLH vaccine
Yoder	1382	Effect of GnRH vaccine on black-tailed prairie dogs
	1563	Transdermal application of a recombinant GnRH vaccine

3/31/2017

On Monday, March 27, I was present during the select agent inspection at the Corwin Springs facility where the USDA Gonacon research is being conducted. The Montana Department of Livestock (MDOL) was present for the inspection to demonstrate our support for the research and to answer any potential questions that the inspector may have had for MDOL.

Present during the inspection were Becky Frey, Ryan Clarke, the inspector, Victoria Guilfoil, and myself.

The following account of statements made during the inspection are to the best of my knowledge and recollection of the inspection:

At the onset of the inspection Dr. Guilfoil indicated to us that the reason for the inspection was that no one was aware that this research was occurring. Becky indicated that there had been an EA on the project that had been published in the federal register. Dr. Guilfoil then alluded that the "Secretary of the Interior called the Acting Secretary of Agriculture to ask about the program". When the Acting Ag Secretary contacted Jack Shere, he was not aware of the research. Jack Shere then reached out to the head of the select agent group, who also did not know about this research.

Dr. Guilfoil then suggested that "when you let your boss get embarrassed, it is a bad thing, and someone needs to have their hand slapped." She then went on to suggest that a "certain individual in Colorado hadn't crossed their T's and dotted their I's." While she expressed this sentiment or something similar multiple times during the interview, at one point she pointedly referred to Jack Ryan in Colorado being at fault for this. She also indicated that Becky and Ryan weren't the ones at fault, the inspection was a fact finding mission.

Dr. Guilfoil explained in what circumstances research is subject to select agent rules and then alluded that Dr. Ryan maybe just "didn't include" or "failed to mention" that there would be seronegative animals involved. This statement felt suggestive that there was underhand methods at work in the implementation of this program.

When MDOL was made aware of the impending inspection and not fully knowing the intent or reason for the inspection, our agency felt it was important to express our support for the research conducted. As a state heavily impacted by the presence of brucellosis in wildlife any research that shows promise in population control or transmission mitigation is of significant value. Dr. Marty Zaluski our state veterinarian was not able to be present for the inspection. To ensure that our support was acknowledged, a signed letter from Dr. Zaluski was presented to Dr. Guilfoil during the investigation. Her response to our letter was laughter and the dismissive statement that it was not important. We would like the record to reflect that we in fact think it is of the utmost importance.

Thank you for the opportunity to provide my account of the inspection. I am happy to answer any questions or provide any clarification I am able to.

Tahnee Szymanski  
Assistant State Veterinarian, Montana Department of Livestock

Bison Work  
August, 2015  
Date:

	Animal ID	Weight	Sex	Ivermectin (ml)	Ear Tags	Blood	Feces	Vaccinations	Comments
+	468	418	F	✓	✓	✓	✓	✓	SKINNY
+	4R6	488	F	✓	✓	✓	✓	✓	NO ADULT INCISORS
+	4R13	409	F	✓	✓	✓	✓	✓	NO adult incisors
+	4R22	550	F	✓	✓	✓	✓	✓	NO ADULT INCISORS
-	3603	784	F	✓	✓	✓	✓	✓	NO ADULT INCISORS
-	4602	615	M	✓	✓	✓	✓	✓	NO adult incisors
-	4606	605	M	✓	✓	✓	✓	✓	
-	4617	460	F	✓	✓	✓		✓	
	420	?	F	✓	✓	✓		✓	8/20
	3R21	702	F	✓	✓	✓	✓	✓	
	<del>627</del>	864	F	✓	✓	✓	✓	✓	
	4R16	480	F	✓	✓	✓	✓	✓	
	4R?	550	F	✓	✓	✓	✓	✓	
	<del>623</del>	910	F	✓	✓	✓	✓	✓	
	626	910	F	✓	✓	✓	✓	✓	
	4R7	616	M	✓	✓	✓	✓	✓	
	4R21	620	F	✓	✓	✓	✓	✓	✓

340003  
003  
344  
952

Ivermectin Dose 1cc/110#  
Ear Tags: Python Magnum  
Blood: 1 Tiger Top and 1 Green Top  
Feces: Johne's PCR +/- Culture  
Vaccinations: Rabies, 9-Way, Cattle Master FP5, Calf Guard

↓  
6.3 ml

## Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

### 2. Action needed:

       Project Completed

       Project Never Initiated

  X   Project On-going/Active: renew as approved

       Project On-going/Active: renew with minor revisions

       Project Not Yet Initiated or is Inactive: renewal requested

Anticipated start date                     

### 3. Protocol Changes

In the upcoming year will you implement any changes to the animal component of the project that differ from those in the original (or subsequent) approval by the IACUC (e.g. changes to animal procedures, number of animals needed, or project objectives)?

Yes        No   X  

### 3. Animal Use and Procedure alternatives since the last IACUC approval

a. Have alternatives to the use of animals become available that could be substituted to achieve specific project aims? Yes        No   X  

b. Have alternatives that are potentially less painful or distressful to animals become available that could be used to achieve specific project aims? Yes        No   X  

If you answered yes to either question, please provide a description below or attach one.

N/A



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3. Number of animals used during second IACUC approval year (enter 0 if in future)	40			
4. Number of animals used during third approval year	42			

**Note:** Additional animals (up to 62) will be collected in winter/spring 2014 to replicate the study as described in the original protocol.

Study Director



Date 12/12/13

Concur

IACUC Chair \_\_\_\_\_

Date \_\_\_\_\_

## Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

Study Title: Evaluation of GonaCon<sup>TM</sup>, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

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Date\_\_\_\_\_

Concur

IACUC Chair \_\_\_\_\_

Date \_\_\_\_\_

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Study Director \_\_\_\_\_ Date\_\_\_\_\_

Concur

IACUC Chair \_\_\_\_\_ Date \_\_\_\_\_

\_\_\_\_\_ Date \_\_\_\_\_

\_\_\_\_\_ Date \_\_\_\_\_

## Study Protocol Renewal/Ammendment

Study Director: Jack Rhyan

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If you answered yes to either question, please provide a description below or attach one.

N/A

Animal usage (please complete the following box):

Commented [pn1]: Becky, can you fill out the first column?

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4. Number of animals used during third approval year	42			

**Note:** Additional animals will be collected (up to 62) in winter/spring 2014 to replicate the study as described in the original protocol.

Commented [pn2]: Based on 104 minus how many we started with in 2011, what would this number be?

Study Director \_\_\_\_\_ Date \_\_\_\_\_

Concur

IACUC Chair \_\_\_\_\_ Date \_\_\_\_\_

## Fuentes, Antonio

---

**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Thursday, June 18, 2015 11:31 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final bru classif.

On 6/18/2015 10:50 AM, Fuentes, Antonio wrote:

Greetings Dr. Houle,

Hope you are enjoying this nice weather.

Would you be able to give us a final classification for two GonaCon Study bison charts.  
Thanks.

Have a good day,  
Antonio

Hi Antonio: Gona Con Study Dr. Ryan Clarke Case#8-439-15. The following are classified as reactors based on positive serological reactions.  
Tubes#s 2 R45,#3 R39,#4 R47,#6 R54,#7 R48, #12 R38,,#15 R53, #17R42,#17 R42,#18 R41,#19 R56,#20 R34,#25 R44, #26 R36, #27, R55, #28 R43, #29 R49,#30 R46, &#31 R51  
Tubes#s 13 Gr 19 & #14 GR 29 are classified as suspects.

Case#8-455-15, Tubes#s 2 R04, #5 R19, #6 R27, #7 R28, #8 R29 are classified as reactors. Tube# 1 R01 is classified as a suspect.

Regards Frank



**Fuentes, Antonio**

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**From:** Frank Houle (b) (6) @gmail.com>  
**Sent:** Friday, June 19, 2015 10:44 AM  
**To:** Fuentes, Antonio  
**Subject:** Re: Final classif. Case No. 8-452-15

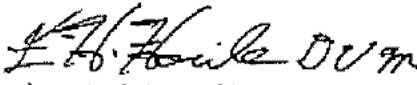
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Designated Brucellosis Epidemiologist

positive serological reactions. Regards

Frank

***NWRC STUDY RECORDS***  
***(Test Substance = 'GnRH')***  
***(March 2, 2011)***

<i>Study Director</i>	<i>QA Number</i>	<i>Title</i>
Felix	533	Use of attenuated Salmonella typhimurium as a live vector for the oral delivery of immunocontraceptive vaccines
Mathies	939	Potential of gonadotropin-releasing hormone antigen for ophidian immunocontraception
	1075	Evaluation of five agents for inhibiting reproduction in the Brown Tree Snake
	1233	Effect of a GnRH vaccine on male brown tree snake reproduction
Miller	451	Development of immunocontraception technology to control reproduction in the coyote (Canis latrans)
	579	Oral contraceptives for the Norway rat
	1396	NWRC adjuvant efficacy testing
Nash	871	Efficacy tests in rabbits for adjuvant and antigen formulations in infertility immunization
	871	Efficacy tests in rabbits for adjuvant and antigen formulations in infertility immunization
	911	Test of GonaCon (GnRH vaccination) treatment in an urban Berkeley, California population of California ground squirrels as a population management tool
Perry	1216	Chemical sterilization of black-tailed deer
Powers	933	Evaluation of Adjuvac emulsion as an alternative to Freund's complete and incomplete adjuvant as a carrier for GNRH-KLH vaccine
Yoder	508	Development and comparison of immunocontraceptive techniques and a competitive cholesterol inhibition technique for control of avian reproduction
	1062	Development of a vaccination protocol for immunization of Japanese Quail

***NWRC STUDY RECORDS***  
***(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

***NWRC STUDY RECORDS***  
***(Test System = 'Bison')***  
***(March 2, 2011)***

<b><i>Study Director</i></b>	<b><i>QA Number</i></b>	<b><i>Title</i></b>
Phillips	1371	Automated species recognition system for controlling animal access to resources: calibration and evaluation for North American species

## Fuentes, Antonio

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**To:** Fuentes, Antonio  
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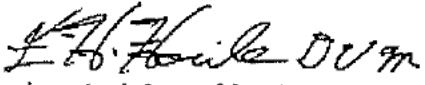
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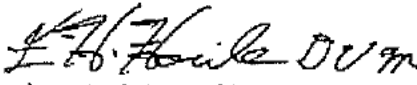
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United States  
Department of  
Agriculture

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease exposure to *Brucella abortus*, the bacteria that can cause -brucellosis. APHIS plans to publish an environmental assessment concerning this project soon. We wanted to notify you of this potential project and are requesting your comments.

One significant way that brucellosis can be spread between infected and uninfected bison happens when infected animals give birth. The materials associated with giving birth contain *Brucella abortus*, and uninfected bison often become exposed to the infected material. The study that APHIS wants to conduct will investigate one way to decrease the potential for this exposure to take place by preventing infected bison from giving birth.

Some of the animals that will be used in the study were captured last spring and the remainder will be captured in upcoming years. Blood samples will be collected from captured bison to test to see if there is evidence of brucellosis infection. Bison that test positive for the presence of brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows, and 8 seronegative bison bulls. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years.

In the proposed study, half of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the GonaCon® vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile from the vaccine, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept in separate areas during the study. Animals will be cared for throughout the study and abortions and births will be monitored. Seronegative bison will be placed with the seropositive GonaCon®-vaccinated animals and with the seropositive non-GonaCon®-vaccinated animals to evaluate transmission of brucellosis. Following each birthing event, all bison will be examined to see if they have produced infected materials that are capable of transmitting *Brucella abortus* to other bison.

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, animals that have tested negative for brucellosis that also meet the requirements for previously-established quarantine use will be placed on tribal or public lands.



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001239

Tribal Leader  
Page 2

| We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at [Terry.W.Clark@aphis.usda.gov](mailto:Terry.W.Clark@aphis.usda.gov) or by telephone at (919) 855-7167.

Sincerely,

John R. Clifford  
Deputy Administrator

National Veterinary Services Laboratories	
Document Title: Summary Report Form for Trips, Meetings, Committees, and Working Groups	
Author/Position: Nancy Clough, Chief of Staff	Document Number: FMA-NVSL-0001.03
Page 1 of 2	Supersedes: NVSLFMAMR01.02

**Summary Report Form for Trips, Meetings, Committees, and Working Groups**

Pauline Nol (Wildlife Epidemiologist) and Samantha Bruce (Saul Wilson Scholar) travelled to Gardiner, MT on June 7, 2015. On June 8, 9, and 10 Dr. Nol and Ms. Bruce assisted with bison handling and sample collection from bison being held at the Bison Quarantine Facility in Corwin Springs, MT. This work is a required component of the protocol associated with the study entitled “Evaluation of GonaCon<sup>TM</sup>, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison”. . Dr. Nol and Ms. Bruce travelled back to Fort Collins, CO on June 10, 2015. This study is part of WiLDIT’s research on management of diseases at the wildlife-livestock interface.

Approved: /s/ Paul Hauer

**Summary Report Form for Trips, Meetings, Committees, and Working Groups**

**Name:** Pauline Nol

**Purpose/Function:** Travel to Gardiner, MT to assist with bison handling and sample collection related to the following study: "Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison". Bison are being held at the Bison Quarantine Facility in Corwin Springs, MT

**Date(s):** June 7-10, 2015

**Location:** Gardiner, MT

**Meeting Title:** NA

**Participant(s)/Attendees:** NA

**Bullet point summary of meeting topics of possible interest/impact to NVSL:** NA

**Feedback requested from Director's Office:** NA

**Meeting proceedings/notes attached:** Yes \_\_\_ No X

**Summary report\* submitted to travel clerk with voucher information:** Yes X No \_\_\_

\*Required to be submitted with travel voucher within 5 business days of return. If no voucher is involved, submit directly to supervisor and Lead Secretary within 3 business days of meeting.

**USAHA Committee on Wildlife Diseases**  
**November 16, 2010, 8:00AM – 12:00 PM**  
**Salon B**  
**Minneapolis Hilton**  
**Minneapolis, Minnesota**

**Agenda**

Dr. Stephen M. Schmitt, Chair and Dr. Colin M. Gillin, Co-Chair

**Bighorn Sheep**

8:00-8:05	Introductory Comments	Steve Schmitt, Colin Gillin
8:05-8:25	Report of the Wild/Domestic Sheep Working Group	Walt Cook
8:25-8:50	Pneumonia in Bighorn Sheep	Sri Subramaniam

**Cervids and Bison**

8:50-9:05	Bovine Tuberculosis in Minnesota Wildlife	Erika Butler
9:05-9:20	CWD National Program	Pat Klein
9:20-9:40	Brucellosis Transmission Dynamics in the Northern GYA	Brant Schumaker
9:40-9:55	Brucellosis Challenges in GYA	Marty Zaluski
9:55-10:10	Brucellosis in Wildlife in the GYA	Mark Drew
10:10-10:25	Hemorrhagic Disease	Mark Ruder

**Wolves, Bats, Wild Birds and Prairie Dogs**

10:25-10:40	Echinococcus in Wolves	Mark Drew
10:40-11:00	White-Nose Syndrome in Bats	Jonathan Sleeman
11:00-11:15	Avian Influenza Research	Justin Brown
11:15-11:30	Oral Plague Vaccine	Tonie Rocke

**Committee Business**

11:30-12:00	Resolutions and Other Committee Business	Steve Schmitt, Colin Gillin
-------------	------------------------------------------	-----------------------------

Animal usage (please complete the following box):

Enter one species in each box and report vertically (if more than 4, list on separate attachment )	Bison			
1. Number approved <u>FOR TOTAL PROJECT</u> on current approval notification <u>plus</u> any subsequent amendments	100			
2. Number of animals used during first IACUC approval year	38			
3. Number of animals used during second IACUC approval year (enter 0 if in future)	20			
4. Number of animals used during third approval year (or to be used if in future)	60			

Note: the 20 animals used in year two were repeat collections of animals also sampled in year one.

Study Director

Jacob Rhyan

Date 12/18/13

Concur

IACUC Chair

Phyllis Clarke

Date 1/2/14

IACUC  
member

Dan Tyers

Date 1/25/14

IACUC  
member

\_\_\_\_\_

Date \_\_\_\_\_

Study Protocol: J Rhyon

Animal usage (please complete the following box);

Enter one species in each box and report vertically (if more than 4, list on separate attachment)	Bison			
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Date 12/18/13

Concur

IACUC Chair

  
P. Ryan ClarkeDate 1/2/14IACUC  
member  
Jerry WiscombDate 1/6/14IACUC  
member

Date \_\_\_\_\_

## WiLDIT Accomplishments FY 2015

### Bison and elk work:

Continued GonaCon studies in southern Colorado and Montana

Assisted CSU in reproductive work

Played key role in preparations for release of YNP genetics bison on public ground to found the Laramie Foothills Conservation Herd (Nov 1 is release date) and arranged to provide bulls with YNP genetics for founder herd at Midewyn Tall Grass Prairie in Illinois. (Relocation occurs mid-October)

Currently conducting challenge study using finely powdered RB51 compounded with montmorilite in mice. If successful, we will try this approach in elk.

Submitted work for patent on DryDart to ARS patent committee. ARS patent committee accepted assignment and submitted application to Patent office. We are continuing to develop DryDart and are starting live animal study to measure immune response to RB51 delivered by DryDart compared to hand vaccination.

Arranged with the states of Colorado and Wyoming to collect up to 20 *Brucella*-positive, pregnant, wild elk cows at feedgrounds in Wyoming in winter 2016, to be transported to Colorado for a natural *Brucella* transmission study.

Conducted study in collaboration with Colorado Parks and Wildlife and Wyoming Game and Fish Commission on efficacy of a Nalbuphine/Azapaperone/Medetomidine drug combination in bison.

### Feral Swine:

Continue vaccine study investigating efficacy of killed oral *Mycobacterium bovis* (Spanish and Michigan strains) in feral swine of Texas origin.

Participated in feral swine ear tag study to determine feasible ear tag weights in the context of eventual application of satellite ear tags.

Investigation of use of volatile organic compounds in breath and feces of swine for detection of *Mycobacterium tuberculosis* complex infection. Collected breath and fecal VOCs from wild boar in Doñana National Park in Spain, September 2015, to be analyzed by collaborators at Roviri i Virgili University in Tarragona, Spain. Will collect VOCs from feral swine in an experimental *M. bovis* challenge in Fall, 2015, also to be analyzed by Roviri i Virgili University.



Visited Texas A and M facility in Kingsville, TX to explore possibilities in collaborative feral swine work with researchers at that university.

Working with Hawaii Department of Agriculture to receive feral swine from Molokai, HI for future testing of tuberculosis vaccines.

## Cattle

Collected breath and fecal samples from Michigan dairy cattle involved in an outbreak of *M. bovis*. Samples were sent to the Technion, Haifa, Israel for volatile organic compound analysis.

## Publications and presentations:

**Rhyan JC**, Tyers D, Zimmer J, Lewandowski K, Hennager S, Young J, Pappert R, Panella A, and Kosoy, O. 2015. Serologic survey of snowshoe hares in the Greater Yellowstone Area for brucellosis, tularemia, and snowshoe hare virus. *J Wildl Dis* 51:769-773.

USAHA Brucellosis Scientific Subcommittee, Research Updates. October 19, 2014.

Presented Research Updates to National Academy of Sciences, Brucellosis Review Panel September 15, 2015

Presented Research Updates to Brucellosis Research Group in Jackson, WY, Sept 24, 2015.

Stahl R.S., Ellis C.K., **P. Nol**, W.R. Waters, M. Palmer, and K.C. VerCauteren. 2015. Fecal volatile organic compound profiles from white-tailed deer (*Odocoileus virginianus*) as indicators of *Mycobacterium bovis* exposure or *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) vaccination. PLoS One 10(6):e0129740.

Fagre, A., K.A. Patyk, **P. Nol**, T. Atwood, K. Hueffer, and C. Duncan. 2015. A review of infectious agents in polar bears (*Ursus maritimus*) and their long-term ecological relevance. Ecohealth. 2015 Mar 20.

Patyk, K.A., C. Duncan, **P. Nol**, C. Sonne, K. Laidre, M. Obbard, Ø Wiig, J. Aars, E. Regehr, L.L. Gustafson, and T. Atwood. Establishing a definition of polar bear (*Ursus maritimus*) health: a guide to research and management activities. 2015. Sci Total Environ. 514:371-8.

Presented update on wild swine tuberculosis on an international scale and participated in a panel discussion. Many Hosts of Mycobacteria VI: Host Specificity and Dynamics of Mycobacterial Disease, March 26-27, 2015, Tulane National Primate Research Center, Covington, Louisiana,

Presented to undergraduate and graduate students on tuberculosis at epidemiology course at Colorado State University

Presented to students and faculty at the Department of Electronics, Electrical and Automatic Engineering at Rovira i Virgili University, Tarragona, Spain on WiLDIT volatile organic compound research.

### **Students/Externs:**

Eight students were accepted as veterinary externs in FY15. These externs were hosted at NWRC by WiLDIT for two to four week blocks. They represented five veterinary schools in the country. One MPH/veterinary student at Colorado State University was co-mentored over the summer by NWRC researchers and WiLDIT researchers. One local high school student interned for WiLDIT over the summer.

WiLDIT has one Saul T. Wilson Scholar from Colorado State University. This student is participating in lyophilized *Brucella* vaccine research in mice.

WiLDIT is supporting and mentoring one MS student at Colorado State University. This student is a DVM and is participating in feral swine tuberculosis vaccine research.

Served on PhD committee for DVM/PhD student studying brucellosis at Colorado State University.

Five undergraduate students from Colorado State University perform animal care and maintenance at the WiLDIT wildlife research facility. These students are supported through a cooperative agreement with the Animal Population Health Institute at Colorado State University.

### **Other:**

Two WiLDIT members participated in HPAI task force in Minnesota, May 3–June 1.

Two WiLDIT members participated in a regulatory veterinary medicine laboratory at Colorado State University.

Produced a material transfer agreement with NEIKER, Spain to use killed *Mycobacterium bovis* vaccine in feral swine.

Established cooperative agreement with the Department of Electronics, Electrical and Automatic Engineering, Rovira i Virgili University, Tarragona, Spain to conduct volatile organic compound analysis on breath and feces to detect tuberculosis in wild boar and feral swine

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to conduct tuberculosis volatile organic compound and molecular research, and support the wildlife research facility.

Established cooperative agreement with the Department of Pathobiological Sciences to conduct tuberculosis vaccine research in feral swine.

Established cooperative agreement with the Department of Clinical Sciences, Colorado State University to support a MS student in research of tuberculosis vaccines in feral swine.

## **WiLDIT Accomplishments FY 2015**

### **Bison and elk work:**

Continued GonaCon studies in southern Colorado and Montana

Assisted CSU in reproductive work

Played key role in preparations for release of YNP genetics bison on public ground to found the Laramie Foothills Conservation Herd (Nov 1 is release date) and arranged to provide bulls with YNP genetics for founder herd at Midewyn Tall Grass Prairie in Illinois. (Relocation occurs mid-October)

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### **Feral Swine:**

### **Publications and presentations:**

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### **Students/Externs:**

### **Other:**

One WiLDIT member participated in HPAI task force in Minnesota, May 3–24.

## **Joint WiLDIT and GYA**

### **Current Projects and Activities Update, August 2012**

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

1. Investigation of seminal shedding of *B. abortus* by Yellowstone bulls in the spring. The study demonstrated shedding of *B. abortus* in the semen of Yellowstone bulls in the spring and evaluated semen quality.
2. Bison Quarantine Feasibility Study. Study demonstrated successful graduation of seronegative, culture negative bison by the diligent use of the bison quarantine protocol published in the UM&R. Collaborators: Montana Department of Fish, Wildlife and Parks.

Projects – Ongoing field/animal work

1. Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means to decrease shedding of *B. abortus* in bison. Project will evaluate shedding of *B. abortus* in contracepted and control bison over 6 years. It will also determine outcomes of calves born to positive cows and evaluate shedding of contracepted cows after fertility returns. Collaborators: APHIS-WS, ARS, NPS.

**Wildlife Livestock Disease Investigations Team**  
**Briefing Paper**  
**9 February 2017**

**Mission of the Wildlife Livestock Disease Investigations Team (WiLDIT): Developing science-based solutions to disease problems at the wildlife/livestock/human interface**

WiLDIT came to being in 1997 and was originally purposed to assist in Greater Yellowstone Area (GYA) issues. In 1999 WiLDIT's duties were expanded to engage in wildlife/domestic animal interface issues in general. WiLDIT oversees a captive wildlife research facility in Fort Collins, CO, that houses wild and domestic hoof stock for controlled studies as needed.

As of 9 February, 2017 WiLDIT staff are as follows:

Jack Rhyan, DVM, MS-Wildlife Pathologist  
Pauline Nol, DVM, MS, PhD-Wildlife Epidemiologist  
Matt McCollum, MS-Wildlife Biologist  
Morgan Wehtje, MS-Wildlife Biologist  
Karl Held-Animal Health Technician  
Samantha Bruce-Saul Wilson Scholar

Project Overviews and Accomplishments

**Brucellosis**

Brucellosis is a disease that cattle, elk, bison, pigs, and humans. In animals it is transmitted when the infected mother has an abortion.

- Bison Quarantine Feasibility Study
  - Successfully developed a method to build herds of Yellowstone bison that are free of brucellosis.
- DryDart-Patent-pending of a dart technology that allows people to use a shotgun as a dart gun. The drugs in the dart are dry so they have a longer shelf life than liquid drugs. This technology was developed to deliver brucellosis vaccines to bison but has other applications.
- Evaluated the use of volatile organic compounds (VOCs) in breath to detect brucellosis in bison. This is a way to remotely detect animals with brucellosis by collecting their breath rather than blood or other body samples. We look for VOCs that are unique in animals that have certain diseases.

**Foot-and-Mouth Disease**

Foot-and-mouth disease (FMD) is caused by a virus that could devastate the US agricultural economy if it entered this country. It affects cattle, sheep, and swine, but its effects on US wildlife were unknown.

- Determined susceptibility of bison, elk, mule deer, and pronghorn to FMD virus and whether these species can transmit the virus to domestic cattle or become long-term carriers.

**Bovine Tuberculosis**

Bovine tuberculosis is a disease very similar to human tuberculosis that affects cattle, swine and many other species of animals including wild animals. This disease also causes illness in humans.

- Evaluation of a human tuberculosis vaccine (BCG) to protect white-tailed deer against bovine tuberculosis
- Evaluation of the use of volatile organic compounds in breath to detect bovine tuberculosis in cattle

Current Projects

**Brucellosis**

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
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- Evaluation of GonaCon™, a contraceptive vaccine, to stop transmission of brucellosis in bison.  
Funding source: Cattle Health
  - Corwin Springs Bison Facility, MT. Collaborators: APHIS/SPRS (lead); WS/National Wildlife Research Center (NWRC)
  - Great Sand Dunes, CO, evaluation of GonaCon™ as a contraceptive in bison. Collaborators (WiLDIT has lead): WS/NWRC; The Nature Conservancy
- Brucellosis infection/transmission dynamics in elk. Brucellosis is spread when animals abort. We put pregnant brucellosis-infected elk in a pen with elk that don't have brucellosis. If a sick elk has an abortion, they may give brucellosis to the other elk. With this work we can learn a lot about the natural disease in elk and we also hope to test brucellosis vaccines by putting brucellosis-infected elk in a pen with vaccinated elk.  
Funding Source: Cattle Health.
  - WiLDIT Wildlife Research Pens, Fort Collins, CO. Collaborators (WiLDIT has lead): Wyoming Game and Fish Department; Agricultural Research Service; Colorado State University
- Development of DryDart technology to deliver brucellosis vaccine to bison.  
Funding source: Cattle Health.
  - National Animal Disease Center (NADC), Ames, IA. Collaborators: Agricultural Research Service, NADC, Bacterial Disease Unit (lead).
- Use of Assisted Reproductive Techniques to Produce Brucellosis-free Bison with Yellowstone Genetics. When genetically important Yellowstone animals that have brucellosis are sent to slaughter, their genetics are lost forever. This study involves making embryos from those animals and putting them in healthy surrogate bison resulting in the birth of healthy Yellowstone bison calves born to bison mothers that don't have brucellosis.  
Funding Source: Cattle Health
  - WiLDIT Wildlife Research Facility. Collaborators: Colorado State University (lead); VS/SPRS
- Development of volatile organic compound (smells) and oral fluid collection studies for feral swine. Looking at a way to detect disease in feral pigs without having to catch them. We collect breath and feces of wild pigs to look at what unique smells are in pigs infected with different diseases. We put out an interesting looking cloth ball, the pigs chew on it and get it wet with saliva. Then we can collect the ball, squeeze out the fluids and test it to see what diseases the pigs have.  
Funding Source: National Feral Swine Initiative
  - Texas A and M University studies. Collaborators (WiLDIT has lead): Texas A and M University; NWRC
  - Field studies. Collaborators (WiLDIT has lead): APHIS/STAS/CEAH; University of Florida; University of Georgia
- Evaluation of killed preparations of *Brucella abortus* in mice. We can take the bacteria that causes brucellosis, kill it and put it in mist or a fine powder that can be breathed in by healthy animals. This may be a way of getting their immune system to protect them from the bacteria.  
Funding source: Cattle Health
  - Colorado State University. Collaborators (WiLDIT has lead): Colorado State University

Wildlife Livestock Disease Investigations Team  
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**Bovine Tuberculosis**

- Evaluation of killed *Mycobacterium bovis* and a vaccine to protect feral swine from bovine tuberculosis.  
Funding Source: National Feral Swine Initiative.
  - WiLDIT Wildlife Research Facility and Colorado State University. Collaborators (WiLDIT as lead): Colorado State University; University Castilla la Mancha, Spain; Neiker Inc., Spain.
- Development of volatile organic compound (VOCs) studies for detection of bovine tuberculosis in feral swine.  
Funding Source: National Feral Swine Initiative.
  - WiLDIT Wildlife Research Facility; Colorado State University; Spain. Collaborators (WiLDIT as lead): Colorado State University; University Castilla la Mancha, Spain; Rovira i Virgili University.

**Other**

- Development of safe and effective immobilization protocols for wild swine. Using drugs to make pigs unconscious is difficult. And wild pigs are dangerous to handle. We are looking at some new drug combinations that may work better and be easier to use in wild swine. These include drugs that have an antidote so you can wake the pigs up when you are done.
  - WiLDIT Wildlife Research Facility. Collaborators (WiLDIT has lead): NWRC; Colorado Parks and Wildlife; Texas A and M University; Wildlife Pharmaceuticals.



#### Proposed:

##### Inactivated *Mycobacterium bovis* vaccine in feral swine

Spanish researchers at IREC have tested an inactivated *M. bovis* strain for efficacy as a vaccine in wild boar against disease caused by *M. bovis* infection. Administered orally, the vaccine is equally as effective as BCG in wild boar. We propose a study to test efficacy of this vaccine in feral swine. A killed vaccine will be much easier to administer in a field situation. The target population in the US for this vaccine would be the *M. bovis*-infected feral swine population on Molokai Island in Hawaii.

##### *Brucella abortus* infection model in elk

*Brucella abortus* is extremely expensive and difficult to work with due to its status as a select agent. In addition, experimental infection models in elk have not been very successful in mimicking natural infection events. We propose to develop a natural infection model for *B. abortus* in elk that would allow test animals to be held in outdoor pens, therefore reducing costs as well as stress on the animals that would otherwise be required to be held in BSL-3 facilities. This study would involve capturing 3 year old pregnant *B. abortus*-infected elk from the wild as well as nonpregnant, 2-3 year old uninfected elk. Infected and uninfected elk will be housed in the same pen and infected elk will be allowed to calve and abort in the pen. The uninfected elk will be thus naturally exposed to infected fetuses. These exposed elk will be bred and held for another year to monitor whether they seroconvert and abort the following calving season. If such a model will work, it will serve as a natural infection model to test efficacy of vaccine candidates being developed for elk.

##### Elk Genome Project

Sequencing of the elk genome would greatly support and expand researchers' efforts to explore and understand the immunological responses elicited by this species to *Brucella abortus* and will aid in development of vaccines against *B. abortus* infection in elk. Collaborators and funders will need to be identified such as ARS/NADC, Texas A and M University, Iowa State University.

##### *Brucella* vaccine studies in elk and feral swine

Explore *B. suis* vaccine candidates in feral swine in an experimental infection model using feral swine from colony established at the APHI/VS animal pens. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Explore *B. abortus* vaccines in elk. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

##### Ecology and epidemiology of brucellosis in elk in the GYA

##### Feral swine modelling etc. working with Ryan and Steve

Commented [PN1]: Elaborate

Commented [PN2]: Elaborate

#### On going:

##### Evaluation of utilizing volatile organic compounds (VOCs) to detect presence of *Brucella* infection in animals

Develop instrumentation that could be utilized in the field to detect brucellosis in wild bison, elk, and feral swine. Study underway is entitled "Detection of volatile organic compounds in bison as tools for detection of brucellosis". Research partner agency: Collaborator: CSU, Technion, Israel, ARS.

Develop instrumentation that could be utilized in the field to detect bovine tuberculosis in white-tailed deer, elk, feral swine, and cattle. Evaluation of utilizing VOCs to detect presence of *M. bovis* infection in animals. Collaborators: ARS, APHIS/WS, CSU, Technion, Israel.

#### Development and evaluation of tuberculosis vaccines and vaccine delivery methods for white-tailed deer and feral swine

Determination of persistence of BCG in feral swine orally vaccinated with BCG. Animal work is completed. Culture of tissues pending. Collaborators: NVSL, CSU

Development of vaccine delivery systems for administration of oral BCG vaccine to wild white-tailed deer. Collaborators: APHIS/WS, ARS, MIDNR

#### Diagnostics for brucellosis and bovine tuberculosis

Use of lipidomics in serologic diagnosis of brucellosis infection in animals. Working with CSU scientists to identify lipids uniquely produced by *Brucella* species in order to

#### Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic

Commented [PN3]: Elaborate

#### Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic

Commented [PN4]: Elaborate

#### Evaluation of brucella vaccines in feral swine

Two vaccine candidates are currently being evaluated in feral swine and domestic swine for protection against *B. suis* infection. This is a nonpregnant animal study and includes barrows and gilts. One vaccine candidate is a field strain rough *B. suis* discovered in South Carolina feral swine by ARS/NADC researchers. The second vaccine is an engineered rough *B. suis* developed by Va Tech which contains a plasmid that expresses GnRH protein.

#### Development of nonlethal methods to eliminate *B. abortus* from bison and elk in the GYA

Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison. Demonstrate efficacy of GnRH vaccine (GonaCon™) in producing infertility, thereby preventing transmission of brucellosis, in bison and elk. Study initiated in May, 2012 in Corwin Springs, MT is entitled and will continue through 2017. Research partner agency: WS. Other collaborators: Idaho F&G, WY G&F, MTFWP, NPS, APHIS/WS, CSU.

Evaluation of GonaCon™, an immunocontraceptive vaccine, in free-ranging bison: A pilot study. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in free-ranging female bison on property owned by The Nature Conservancy (TNC) and managed by Zapata Partners (Medano-Zapata Ranch). The property is adjacent to Great Sand Dunes National Park in south central Colorado. Study

initiated in November 2011. Ten bison cows were vaccinated with GonaCon™ and ten bison serve as controls.

Embryo Transfer as disease mitigation strategy in bison.

Collaborators: CSU, WCS, NVSL

International work

FADs

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**On going:**

- Evaluation of volatile organic compounds and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals
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- Evaluation of brucellosis vaccines in feral swine
- Development of nonlethal methods to eliminate *Brucella abortus* from bison and elk in the GYA
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**Proposed Short Term**

- Inactivated *Mycobacterium bovis* vaccine in feral swine
- *Brucella abortus* infection model in elk

**Proposed Long Term:**

- Elk Genome Project
- *Brucella* vaccine studies in elk and feral swine
- Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic
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- FADs
- International work

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**On going:**

**Evaluation of volatile organic compounds (VOCs) and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals**

Develop instrumentation that could be utilized in the field to detect brucellosis in wild bison, elk, and feral swine. Study underway is entitled "Detection of volatile organic compounds in bison as tools for detection of brucellosis". Collaborators: CSU, Technion, Israel, ARS.

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Working with CSU scientists to identify lipids uniquely produced by *Brucella* species in order to identify *Brucella*-infected animals as well as distinguish the particular species of *Brucella*. This work will lead to the development of ELISA tests to serologically distinguish between *Brucella* spp. and to differentiate them from *Yersinia enterocolitica* 0:9 infection. Collaborators: CSU, USDA/ARS.

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Investigate the seroprevalence of *Brucella* spp. antibodies in grizzly bears and polar bears in Alaska and the Greater Yellowstone Area. Data will be obtained from archived samples and samples to be collected during future field capture events. Collaborators: USGS, State of Alaska, NVSL, CEAH, State of MT

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exposed to infected fetuses, placentas, and/or discharges. These exposed elk will be bred and held for another year to monitor whether they seroconvert and abort the following calving season. If this approach results in the seroconversion and infection of a significant number of initially uninfected animals, it will serve as a natural infection model to test efficacy of vaccine candidates being developed for elk. This strategy could be thought of as a controlled field trial in confinement. The large cost benefit of using this model would allow greatly accelerated testing of candidate vaccines for brucellosis in elk. Collaborators for this project will include WY Game and Fish and ARS/NADC.

## **Proposed Long Term:**

### **Elk Genome Project**

Sequencing, assembly, and annotation of the elk genome would greatly support and expand researchers' efforts to explore and understand the immunological responses elicited by this species to *B. abortus* and will aid in development of vaccines against *B. abortus* infection in elk. Additionally, this valuable knowledge will support vaccine and diagnostics development in the context of other elk diseases as well. Collaborators and funders will need to be identified such as ARS/NADC, Texas A and M University, Iowa State University.

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Continued exploration of *B. abortus* vaccines in elk. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

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Human populations in the Arctic ecosystem are potentially exposed to zoonotic agents through subsistence hunting practices which include raw meat preparation and consumption. Marine mammal *Brucella* spp. and *B. suis* biovar 4 (found in terrestrial animals especially caribou) as well as other agents, such as *Coxiella* spp., exist on the arctic landscape and can cause disease in humans. In the event of a human diagnosed with brucellosis, steps to determine the *Brucella* species involved are generally not taken. Although the epidemiology and ecology of *B. suis* in caribou and reindeer is relatively well understood, we have very limited information on marine mammal *Brucella* species. We intend to submit a grant proposing to investigate the epidemiology of marine mammal *Brucellae* and the role they play in Arctic wildlife health as well as human health. Collaborators: Varied but may include NVSL, CSU, State of AK, USGS, University of AK, Burrow of Barrow, AK, University of Calgary.

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**FADs**

Initiate studies examining the pathogenesis of Rift Valley fever in potentially susceptible North American wildlife such as white-tailed deer, bison, and elk

Evaluate foot and mouth disease vaccines for use in feral swine and white-tailed deer.

**International work**

Access to *Brucella* and *M. bovis*-infected animals in the United States is limited to experimental infection studies and the occasional outbreak in cattle and captive cervids. Few opportunities arise that allow researchers to collect samples from naturally infected animals in adequate numbers. Collaborations with colleagues in other countries such as Mexico could make relatively large numbers of infected animals accessible for testing breath samples from cattle for *M. bovis* or *B. abortus*-specific VOCs.



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**Work Plan FY 2013**

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**Work Plan FY 2013**

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**On going:**

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- Development and evaluation of tuberculosis vaccines and vaccine delivery methods for white-tailed deer and feral swine **2005-2017**
- Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic 2012-2015
- Evaluation of brucellosis vaccines in feral swine **2012-**
- Development of nonlethal methods to eliminate *Brucella abortus* from bison and elk in the GYA **2004-**
- Embryo Transfer as disease mitigation strategy in bison **2011-**

**Proposed Short Term**

- Inactivated *Mycobacterium bovis* vaccine in feral swine **2013-2014**
- *Brucella abortus* infection model in elk **2013-2014**

**Proposed Long Term:**

- Elk Genome Project **2013-**
- *Brucella* vaccine studies in elk and feral swine **2012**
- Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic **2012-2015**
- Ecology of diseases in feral swine in the United States **2013-**
- FADs **2014-**
- International work **2013-**

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Wildlife Livestock Disease Investigations Team  
Work Plan FY 2013**

**On going:**

**Evaluation of volatile organic compounds (VOCs) and bacterial nucleic acids to detect presence of *Brucella* and *Mycobacteria* infection in animals**

Develop instrumentation that could be utilized in the field to detect brucellosis in wild bison, elk, and feral swine. Study underway is entitled "Detection of volatile organic compounds in bison as tools for detection of brucellosis". Collaborators: CSU, Technion, Israel, ARS.

Develop instrumentation that could be utilized in the field to detect bovine tuberculosis in white-tailed deer, elk, feral swine, and cattle. Study underway is entitled "Detection of volatile organic compounds and bacterial nucleic acids in animals as tools for diagnosis of tuberculosis". Collaborators: ARS, APHIS/WS, CSU, Technion, Israel.

**Budget:**

**\$40,000**

Binational Agricultural Research and Development Fund Grant was submitted for *M. bovis* and *M. avium* paratuberculosis in cattle for \$284,000 between CSU and the Technion, Israel.

**Development and evaluation of tuberculosis vaccines and vaccine delivery methods for white-tailed deer and feral swine**

Determination of persistence of BCG in feral swine orally vaccinated with BCG. Animal work is completed. Culture of tissues pending. Collaborators: NVSL, CSU

**Budget** (Animal feed and maintenance for 9 months)

**\$7,000**

Development of vaccine delivery systems for administration of oral BCG vaccine to wild white-tailed deer. Collaborators: APHIS/WS, ARS, MIDNR

**Budget** (Travel)

**\$1,500**

**Lipidomics as a diagnostic method for brucellosis and bovine tuberculosis**

Working with CSU scientists to identify lipids uniquely produced by *Brucella* species in order to identify *Brucella*-infected animals as well as distinguish the particular species of *Brucella*. This work will lead to the development of ELISA tests to serologically distinguish between *Brucella* spp. and to differentiate them from *Yersinia enterocolitica* 0:9 infection. Collaborators: CSU, USDA/ARS.

**Budget**

To be determined

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**Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic**

Investigate the seroprevalence of *Brucella* spp. antibodies in grizzly bears and polar bears in Alaska and the Greater Yellowstone Area. Data will be obtained from archived samples and samples to be collected during future field capture events. Collaborators: USGS, State of Alaska, NVSL, CEAH, State of MT

NPRB grant being submitted for Alaskan polar bear work

\$84,000

**Evaluation of brucellosis vaccines in feral swine**

Two vaccine candidates are currently being evaluated in feral swine and domestic swine for protection against *B. suis* infection. This is a nonpregnant animal study and includes barrows and gilts. One vaccine candidate is a field strain rough *B. suis* discovered in South Carolina feral swine by ARS/NADC researchers. The second vaccine is an engineered rough *B. suis* developed by Va Tech which contains a plasmid that expresses GnRH protein. Collaborators: VA Tech, USDA/ARS.

Budget (Farrowing, maintenance, travel)

**\$4,000**

**Development of nonlethal methods to eliminate *Brucella abortus* from bison and elk in the GYA**

Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison. Demonstrate efficacy of GnRH vaccine (GonaCon™) in producing infertility, thereby preventing transmission of brucellosis, in bison and elk. Study initiated in May, 2012 in Corwin Springs, MT is entitled and will continue through 2017. Research partner agency: WS. Other collaborators: Idaho F&G, WY G&F, MTFWP, NPS, APHIS/WS, CSU.

Budget (Travel, Feed and Maintenance, Drugs, Darts, Lease)

Commented [pn1]: Cost??

Evaluation of GonaCon™, an immunocontraceptive vaccine, in free-ranging bison: A pilot study. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in free-ranging female bison on property owned by The Nature Conservancy (TNC) and managed by Zapata Partners (Medano-Zapata Ranch). The property is adjacent to Great Sand Dunes National Park in south central Colorado. Study initiated in November 2011. Ten bison cows were vaccinated with GonaCon™ and ten bison serve as controls.

Budget

**\$3,500**

Commented [pn2]: I have really no idea what this has cost us

**Embryo Transfer as disease mitigation strategy in bison**

Development of an effective embryo transfer technique in bison in order to produce disease-free offspring derived from bison with various infections such as brucellosis and paratuberculosis. Collaborators: CSU, WCS, NVSL



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Budget (Tissue handling and laboratory)

\$

Commented [pn3]: Cost?

**Proposed Short Term**

**Inactivated *Mycobacterium bovis* vaccine in feral swine**

Spanish researchers at IREC have tested an inactivated *M. bovis* strain for efficacy as a vaccine in wild boar against disease caused by *M. bovis* infection. Administered orally, the vaccine is equally as effective as BCG in wild boar. We propose a study to test efficacy of this vaccine in feral swine. A killed vaccine will be much easier to administer in a field situation. The target population in the US for this vaccine would be the *M. bovis*-infected feral swine population on Molokai Island in Hawaii.

Budget (Purchase and shipment of feral piglets from Hawaii, Feed and maintenance of animals (non-BSL3), Animal Care, Animal per diem BSL 3, Laboratory/Immunology, Tissue Culture, Histologic Preparation

**\$31,150**

***Brucella abortus* infection model in elk**

*Brucella abortus* is extremely expensive and difficult to work with due to its status as a select agent. In addition, experimental infection models in elk have not been very successful in mimicking natural infection events. We propose to develop a natural infection model for *B. abortus* in elk that would allow test animals to be held in outdoor pens, therefore reducing costs as well as stress on the animals that would otherwise be required to be held in BSL-3 facilities. This study would involve capturing 3 year old pregnant *B. abortus*-infected elk from the wild as well as nonpregnant, 2-3 year old uninfected elk. Infected and uninfected elk will be housed in the same double-fenced paddock and infected elk will be allowed to calve and abort in the pen. The uninfected elk will be thus naturally exposed to infected fetuses, placentas, and/or discharges. These exposed elk will be bred and held for another year to monitor whether they seroconvert and abort the following calving season. If this approach results in the seroconversion and infection of a significant number of initially uninfected animals, it will serve as a natural infection model to test efficacy of vaccine candidates being developed for elk. This strategy could be thought of as a controlled field trial in confinement. The large cost benefit of using this model would allow greatly accelerated testing of candidate vaccines for brucellosis in elk. Collaborators for this project will include WY Game and Fish and ARS/NADC.

Budget (Travel, Capture, Housing, Feed, 17 months)

Commented [pn4]: Talk to Kreeger

**Proposed Long Term:**

**Elk Genome Project**

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Wildlife Livestock Disease Investigations Team  
Work Plan FY 2013**

Sequencing, assembly, and annotation of the elk genome would greatly support and expand researchers' efforts to explore and understand the immunological responses elicited by this species to *B. abortus* and will aid in development of vaccines against *B. abortus* infection in elk. Additionally, this valuable knowledge will support vaccine and diagnostics development in the context of other elk diseases as well. Collaborators and funders will need to be identified such as ARS/NADC, Texas A and M University, Iowa State University.

Budget (Sequencing Assembly and Annotation) **\$120,000**

Will seek collaborators and other support

**Brucella vaccine studies in elk and feral swine**

Continued exploration of *B. suis* vaccine candidates in feral swine in an experimental infection model using feral swine from a breeding colony established at the APHI/VS animal pens. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Continued exploration of *B. abortus* vaccines in elk. Collaborators include ARS/NADC, Va Tech University, APHIS/WS

Budget To be determined

**Ecology and epidemiology of brucellosis in marine mammal species and humans in the Arctic**

Human populations in the Arctic ecosystem are potentially exposed to zoonotic agents through subsistence hunting practices which include raw meat preparation and consumption. Marine mammal *Brucella* spp. and *B. suis* biovar 4 (found in terrestrial animals especially caribou) as well as other agents, such as *Coxiella* spp., exist on the arctic landscape and can cause disease in humans. In the event of a human diagnosed with brucellosis, steps to determine the *Brucella* species involved are generally not taken. Although the epidemiology and ecology of *B. suis* in caribou and reindeer is relatively well understood, we have very limited information on marine mammal *Brucella* species. We intend to submit a grant proposing to investigate the epidemiology of marine mammal *Brucellae* and the role they play in Arctic wildlife health as well as human health. Collaborators: Varied but may include NVSL, CSU, State of AK, USGS, University of AK, Burrow of Barrow, AK, University of Calgary.

Budget To be determined

**Ecology of diseases in feral swine in the United States**

Feral swine populations throughout the United States are expanding rapidly and certain swine diseases of economic importance are therefore expanding as well within these populations. We propose to work in collaboration with the Wildlife/Livestock Disease Unit of CEAH to investigate ranges and movements of feral swine populations, estimate disease prevalence, evaluate certain disease

Commented [pn5]: Cost of raising and maintaining pigs

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management strategies, such as vaccination, and incorporate acquired information into disease models. Diseases of interest will include those already existing in the feral swine populations (swine brucellosis, tularemia, pseudorabies) as well as FADs (foot and mouth disease, classical swine fever).

**FADs**

Initiate studies examining the pathogenesis of Rift Valley fever in potentially susceptible North American wildlife such as white-tailed deer, bison, and elk

**Budget**

To be determined

Evaluate foot and mouth disease vaccines for use in feral swine and white-tailed deer.

**Budget**

To be determined

**International work**

Access to *Brucella* and *M. bovis*-infected animals in the United States is limited to experimental infection studies and the occasional outbreak in cattle and captive cervids. Few opportunities arise that allow researchers to collect samples from naturally infected animals in adequate numbers. Collaborations with colleagues in other countries such as Mexico could make relatively large numbers of infected animals accessible for testing breath samples from cattle for *M. bovis* or *B. abortus*-specific VOCs. Budget

To be determined

# USDA, APHIS, VS, STAS



## Wildlife/Livestock Disease Investigations Team (WiLDIT)

Portfolio 2013

*Developing science-based solutions to disease problems at the  
wildlife/livestock/human interface*

Portfolio  
Wildlife/Livestock Disease  
Investigations Team  
2013

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## **Wildlife/Livestock Disease Investigations Team (WiLDIT)**

### **Veterinary Services**

#### **National Wildlife Research Center, Fort Collins, CO**

#### **Mission**

*Developing science-based solutions to disease problems at the wildlife/livestock interface*

#### **Administrative History**

In 1997, following the '96/97 winter when over 1000 bison from Yellowstone National Park died or were sent to slaughter, Dr. Joan Arnoldi, Deputy Administrator of APHIS/VS created the first position of WiLDIT under the Western Regional Director, Dr. Bob Nervig. The position was located at the NWRC as part of the "One APHIS" concept. The purpose of the position was to help with GYA wildlife issues including continuing research projects begun in 1995. In 1999, when the Regional Director, Dr. Bill Buish, went to NVSL, Dr. Arnoldi placed the WiLDIT position under the VS Deputy's supervision and expanded the duties to include engagement in wildlife/domestic animal interface and game farm disease issues in which VS was involved. In 2000, Dr. Alfonso Torres replaced Dr. Arnoldi and reorganized his staff. Dr. Torres the position under Dr. Mike Gilsdorf of the National Animal Health Program. In 2001, Dr. Torres approved WiLDIT to begin work on foot and mouth disease (FMD) in North American wildlife. In 2007, with Dr. Gilsdorf's retirement, National Animal Health Program was reorganized and WiLDIT was placed under Dr. Jerry Diemer, the Assistant Regional Director of the Western Region and leader of the Greater Yellowstone Area core team. Upon Dr. Diemer's retirement in 2009, WiLDIT was placed under Dr. Brian McCluskey, Regional Director of the Western Region. In 2011, Dr. Don Herriot became Assistant Regional Director of the Western Region and oversaw WiLDIT activities until 2013, when VS reorganized. WiLDIT currently resides in the Science, Technology, and Analysis Services group of Veterinary Services and is supervised by Dr. Suelee Robbe-Austerman at the National Veterinary Services Laboratories.

## Staff

Jack Rhyan, DVM, MS-Wildlife Pathologist/Team Leader

Pauline Nol, DVM, MS, PhD-Wildlife Epidemiologist

Matt McCollum, MS-Wildlife Biologist

Karl Held-Animal Health Technician

Kyle Kelly-APHIS Pathways Student Intern

## Activity Areas

**1. Developmental Work** - Coordinate and/or conduct developmental work to address VS-specific problem areas, i.e. vaccine development for wildlife (brucellosis, TB, FMD), test disease diagnostics and detection methods for wildlife (i.e. volatile organic compounds, oral fluids, infrared imaging technology, molecular methods), strategies to eliminate brucellosis from GYA wildlife (i.e., oral vaccination, immunocontraception). Collaborators include, but are not limited to, APHIS-VS, ARS, APHIS-WS, NPS, USGS, Colorado Division of Parks and Wildlife, Colorado State University, Technion-Israel, IREC-Spain, The Nature Conservancy, and the Wildlife Conservation Society.

**2. Consultation** – Provide advice and consultation to Agency on interface disease issues; serve as liaisons with WS; serve as liaisons with State and Federal wildlife agencies and NGO's.

**3. Training** – Serve as training resource for agencies and universities concerning interface diseases (i.e. wildlife disease instruction at CSU, *Brucella* epi, and FAD courses).

**4. Workshops/Meetings** – Participate in and present information and research findings to various relevant audiences; develop collaborations with colleagues.

**5. One Health** – Conduct developmental work, trainings, and consultations, assist with One Health Office operations, and participate in meetings that apply directly to One Health. Serve as assistant liaisons for VS One Health Office.

## Justification

**Why in Veterinary Services?** In 1997, when the first position was established, Wildlife Services was not involved in any disease work with the exception of rabies. Since then, WS has developed a disease program including work on avian influenza, tuberculosis, West Nile virus, and chronic wasting disease, in addition to rabies. WiLDIT has consistently liaised with WS in the development of the WS work and routinely collaborates with WS personnel in laboratory

research and field work. This arrangement works well for both sister agencies. Examples of collaborative field work include: volatile organic compounds for disease detection, vaccine development for bovine TB in wildlife, immunocontraception for disease management, benefit-cost analyses of wildlife/livestock disease management.

**Why in Fort Collins?** WiLDIT's location at the NWRC is beneficial to both VS and WS.

Additionally, the Fort Collins location allows frequent collaboration with other VS, ARS, CSU, DOI, and State of Colorado domestic animal and wildlife disease experts. A valuable continuing relationship for the WiLDIT is that with the Animal Population Health Institute at CSU.

Through a Memorandum of Understanding the two entities share a wildlife research facility and routinely collaborate on projects. The APhi laboratory is a valuable resource for conducting molecular and microbiological work and APhi provides funding for students to work at the wildlife research facility. In addition, WiLDIT and the Animal Reproduction and Biotechnology Laboratory at CSU have also put into place an MOU concerning collaborative work with embryo transfer technologies in bison for brucellosis management.

## **Students**

In conjunction with Colorado State University, WiLDIT plays a strong role in the education and training of numerous students by providing employment and volunteer opportunities, internship/externship programs, project work for special studies students and graduate students, as well as participation on graduate committees. In the last ten years, through cooperative agreements and grants with CSU, WiLDIT has been able to employ over 20 students as animal care and laboratory technicians, many of whom have gone on to veterinary school.

Since its inception in 1997, WiLDIT has hosted nearly 50 student interns and externs from throughout the United States as well as from Canada, Europe, and South America, eager to learn about research in wildlife, livestock and human health in both government and academic settings. WiLDIT works with the various other wildlife, livestock, and public health agencies in the area in order to coordinate a diverse and comprehensive experience for veterinary, graduate, and undergraduate students.



### **Academic Affiliations**

Two WiLDIT members (Rhyon, Nol) are affiliated faculty in the Department of Clinical Sciences and Department of Biomedical Sciences at Colorado State University and have served on nine graduate student committees.

### **CSU/APHIS Wildlife Research Facility**

The CSU/APHIS Wildlife Research Facility occupies approximately 6.5 acres and is located on the Colorado State University Foothills Campus adjacent to the National Wildlife Research Center in Fort Collins, Colorado. The facility consists of multiple large paddocks with 8-foot high walls, while the entire perimeter of the site is surrounded by a 10-foot high wire fence with added wire to exclude small predators. This facility contains handling equipment for deer, bison, elk, bighorn sheep, feral swine, and other ungulate species. Security is provided by USDA/APHIS, National Wildlife Research Center and CSU security personnel. The animals and facility are maintained by a full time animal care staff and an attending veterinarian. The majority of projects conducted at the facility are approved by the Colorado State University Animal Care and Use Committee. Additionally, some ACUCs are approved by NWRC's IACUC and Quality Assurance staff and some are approved by the Bison Quarantine Facility IACUC in Bozeman, Montana.

## Wildlife/Livestock Disease Investigations Team

### Summary of Ongoing and Future Projects

#### Ongoing Projects and Completed Projects (2010-2013)

1. GonaCon™ study to determine if immunocontraception decreases shedding of *B. abortus* in bison. Study started in 2011 at Bison Quarantine Facility in Corwin Springs, MT. Animal work ongoing. Collaborators: WS, NVSL, NPS
2. 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: WS, USGS- BRD, TNC.
3. Develop embryo transfer technology for disease mitigation in bison. A portion of this work investigates the collection and use of ova and semen from naturally-infected bison to potentially result in *B. abortus* negative offspring. Animal work ongoing. Collaborators: CSU, Wildlife Conservation Society
4. 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 in spray-dried form. Animal work ongoing. Collaborators: WS, NVSL, ARS, CSU
5. Continue development of methods for detection of diseases in ruminants using volatile organic compounds in breath and feces. FY 14 and after. Collaborators: WS, NVSL, ARS, CSU, Technion-Israel
6. Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic. Prospective and retrospective study of prevalence of brucellosis in polar bears 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. Two manuscripts in preparation and one pending.
7. 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
8. 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 demonstrated shedding of *B. abortus* in bison semen. Third breeding trial study planned. A disease reduction strategy based on the use of an immunocontraceptive would not be effective in preventing shedding if venereal transmission is common in bison.

6. 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. . Collaborators: APHIS-WS; APHIS-VS-CEAH. Manuscript in preparation
7. 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. Collaborators: ARS, CSU, Virginia Tech. Manuscript in preparation.
8. Detection of volatile organic compounds (VOCs) in breath of animals infected with TB and brucellosis. Studies investigate effectiveness of VOC detection in breath and feces as a screening/diagnostic tool for TB and brucellosis in cattle, bison, and white-tailed deer. Collaborators: APHIS-WS, ARS, CSU, Technion-Israel. Two manuscripts published and one in preparation.
9. Development of breath collection device for feral swine in order to remotely detect diseases in swine using volatile organic compounds. Developmental work ongoing
10. BCG tissue persistence in feral swine. Study designed to determine tissue clearance of orally administered BCG vaccine in feral swine. Study needed prior to eventual BCG field trial on Molokai. Collaborators: ARS, NVSL, CSU. Manuscript in preparation.
11. Transmission of BCG among white-tailed deer and cattle following oral vaccination of deer. Some deer comingled with vaccinates became skin test positive. Cattle sharing facilities and feeders but not in contact with vaccinates remained negative. Study is one of several needed prior to field trial of vaccine in Michigan. Collaborators: ARS, NVSL, CSU. Manuscript published.
12. Quarantine Feasibility Study. Determine feasibility of quarantine procedures of bison calves from Yellowstone National Park for conservation of brucellosis free bison. Collaborators: MT FWP, MT DL, DOI/NPSe/YNP, NVSL. Manuscript in press

## Future Projects

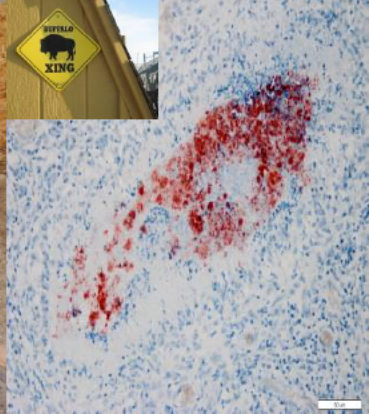
1. *Brucella abortus* infection/transmission model in elk. Project to start in winter/early spring 2014. A natural infection/transmission model will be tested in *B. abortus*-infected wild elk obtained from the GYA. If successful, this model will be used for subsequent vaccine studies in this species. Collaborators: ARS, WY GFC, CSU
2. Applying RB51 as a management tool to control brucellosis in free-ranging bison in Yellowstone National Park; a field trial. Discussion stage. Collaborators: ARS, NPS
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: WS, CSU, Technion-Israel, ARS
7. 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 feral swine attractants for collection device. This is part of a larger research direction to investigate the use of temporary feeder sites on which to conduct disease surveillance (using thermal imaging, VOC analyses, and oral fluid collection for microbiologic and antibody testing) and disease management techniques (vaccination; euthanasia, etc). Ongoing. Collaborators: APHIS-WS, CSU
8. Efficacy of Adenovirus-based FMD vaccines in feral swine. This study is to determine antibody response and efficacy against challenge of FMD vaccines in feral swine of Texas origin. Discussion stage. Collaborators: DHS, VS, ARS
9. Develop cost benefit analysis of using the bioeconomic decision model described for managing brucellosis in wildlife in the Greater Yellowstone Area. Discussion/data collection stage. Collaborators: WS; VS/CEAH





# Ongoing Projects



## **Evaluation of immunocontraception as a means of decreasing shedding of *Brucella abortus* in bison**

**WiLDIT Staff:** Jack Rhyan, Matt Mcollum, Pauline Nol, Karl Held

**Collaborating Agencies:** USDA/APHIS/VS/SPRS and NVSL;  
USDA/APHIS/WS/NWRC, USDA/ARS/NADC; National Park Service

**Background:** Brucellosis has been endemic in the GYA bison since at least 1917. Since the 1940's there has been sporadic unsuccessful pressure on the NPS to eradicate the disease. In recent years, that pressure has been resisted on the premise that the only tool to be used I test-and-slaughter and that today's public sensitivity would not allow it. Because of this, we are demonstrating alternate non-lethal techniques to stop transmission. The cornerstone to this strategy is the use of contraception.

**Study design:** 2011-2013-A total of 50 young female bison (seronegative and seropositive) were captured in late winter/spring 2011, and 2012 as part of the ongoing Interagency Bison Management Plan and transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana. In spring 2012, duplicates of 15 seropositive cows and 4 seronegative cows were put into two replicate test pastures. GonaCon<sup>TM</sup> vaccine (containing 3000µg in 3 ml adjuvant) was delivered to the seropositive cows in one of the pastures intramuscularly (1 ½ ml on either hip). Cows were exposed to bulls in summer/fall. Reproductive outcomes for all cows were monitored. Within five days of abortion/parturition, cow and calf (when possible) were sampled to monitor for evidence of shedding and evidence of *B. abortus* transmission in the case of originally seronegative animals.

2014-2019-Attempts will be made to capture enough bison in 2014-2015 in order to replicate the above experiment. Monitoring of the first cohort will continue until 2017 and the next group will monitored until 2019. At the end of the study, all seropositive animals will be euthanized necropsied and examined for evidence of *B. abortus* infection and lesions in various tissues.

**Results and Conclusions:** Preliminary-Of 12 seropositive cows, there were 2 *Brucella*-positive abortions, 2 *Brucella* -positive calvings, and 8 *Brucella*-negative calves.

**Publications:** Pending

## **Evaluation of GonaCon<sup>TM</sup>, an immunocontraceptive vaccine, in free-ranging bison: A pilot study**

**WiLDIT Staff:** Jack Rhyan, Matt McCollum, Pauline Nol

**Collaborating Agencies:** USDA/ARS/NADC, USDA/APHIS/WS/ NWRC, Virginia Polytechnic Institute and State University

**Background:** Immunocontraception is a tool that could be effectively used for reproductive, genetic, and disease management of wild bison herds throughout North America. GonaCon<sup>TM</sup>, an immunocontraceptive vaccine containing GnRH, approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. Its use has been proposed as a nonlethal method of managing bison populations

**Study Design:** In November, 2011, 20 adult female bison were selected for inclusion in this study. Ten animals were vaccinated with 3000 ug GnRH in a 3 ml volume via intramuscular injection, 1.5 ml vaccine will be administered in either side of the hip. Ten bison are designated non-vaccinated controls. All study bison were fitted with color-coded collars. In summer of 2012 and 2013 attempts were made to observe the study animals to determine presence or absence of calves. In November, 2012 and 2013 during routine handling of the entire herd, study animals were bled for antibody titers to GnRH and checked for evidence of a calf (udder development) and pregnancy. All study animals range freely with the remaining herd of 2000 head.

**Results and Conclusions:** Pending

**Publications:** Pending



## **Efficacy of nebulized killed *Brucella abortus* against virulent *B. abortus* infection in mice:**

**WiLDIT Staff:** P. Nol, J. Rhyan, Kyle Kelly

**Collaborating Agencies:** USDA/APHIS/ VS/ NVSL, USDA/ARS/NADC, USDA/APHIS/WS/ NWRC

**Background:** Wildlife vaccine discovery and implementation of a vaccine program pose many challenges, one of which is developing a safe and effective delivery strategy in the field. Ensuring adequate exposure to vaccine to as many animals as possible, maintaining the integrity of the vaccine under field conditions, and the overall safety of the vaccine in both target and non-target species are issues that must be addressed. Preparation methods, such as spray-drying can protect the integrity of the vaccines, and can maintain the viability of live vaccines exposed to unfavorable conditions. Use of killed, DNA, or antigen vaccines, as opposed to live bacterial or viral vaccines, would be advantageous in a wildlife setting, as concerns over vaccine viability and exposure of non-target animals and humans to live agents would be greatly diminished. Utilizing oral or respiratory routes for vaccine delivery in the field precludes the need for individual hand vaccination, darting, or shooting of biobullets and allows passive administration of the vaccine to multiple animals. Aerosolized vaccines, in liquid or dry powder forms, can have advantages over conventional parenteral vaccines or oral vaccines as they come in contact with mucous membranes of the oronasal cavity, lungs and gastrointestinal tract, which are also the routes of entry for *Brucella* and *Mycobacterium* species.

This study is to determine if killed preparations of *B. abortus* administered via nebulizer multiple times to mice will elicit antibody responses to *B. abortus* antigen and induce protection against challenge with virulent *B. abortus*. Subsequent studies will test spray-dried preparations of killed *B. abortus* and *B. suis* vaccines as well as tuberculosis vaccines.

**Study Design:** 100-130 female BALB/c AnNHsD mice will be vaccinated with a GYA-acquired strain of *B. abortus* killed via three different methods to test possible differences in efficacy associated with the killing methods. Each treatment group of mice (n=15) will be exposed to one type of killed product via nebulizer on varying days depending on the treatment assigned. Eight weeks after vaccination, mice will be challenged with virulent *B. abortus* acquired from the GYA. Mice will be euthanized and cultured two weeks post-challenge.

**Results and Conclusions:** Pending

**Publications:** Pending



## **Detection of volatile organic compounds in cattle naturally infected with *Mycobacterium bovis*.**

**WiLDIT Staff:** Jack Rhyan, Pauline Nol, Matt McCollum

**Collaborating Agencies:** USDA/APHIS/WS/ NWRC, Technion, Israel

**Background:** An emerging approach for diagnosing infectious disease at its earliest stages relies on volatile organic compounds (VOCs) that are emitted from the infectious agent and/or the host. This study explored the utility of breath testing for the detection of *M. bovis* infection in cattle.

**Study Design:** Breath samples were collected and tested from 14 cattle from an *M. bovis*-infected dairy in the southern part of the state of Colorado, USA. Ten of these animals were identified as bTB-positive based on conventional tests. Four animals from the same dairy were deemed bTB-negative and were used as on-farm negative controls. Thirteen cattle from two bTB-negative dairies located in northern Colorado were also tested and served as off-farm negative controls. Breath samples from the cattle were collected on Tenax sorbent material and sent to Technion for GCMS and electronic nose (NA-NOSE) analysis.

**Results and Conclusions:** The NA-NOSE system successfully identified all *M. bovis*-infected animals, while 21% of the not infected animals were classified as *M. bovis*-infected. This technique could form the basis for a real-time cattle monitoring system that allows efficient and non-invasive screening for new *M. bovis* infections on dairy farms.

### **Publications:**

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

## **Detection of volatile organic compounds in bison naturally exposed to *Brucella abortus* 2012-2013.**

**WiLDIT Staff:** Jack Rhyan, Pauline Nol, Matt McCollum

**Collaborating Agencies:** Technion, Israel, USDA/APHIS/WS/ NWRC

**Background:** An emerging approach for diagnosing infectious disease at its earliest stages relies on volatile organic compounds (VOCs) that are emitted from the infectious agent and/or the host. This study explored the utility of breath testing for the detection of *B. abortus* infection in bison.

**Study Design:** Breath samples from 20 *Brucella*-seropositive bison and 18 controls were chemically analyzed with GCMS and NA-NOSE. .

**Results and Conclusions:** The analysis demonstrated statistically significant differences in the concentration profiles of five VOCs. The NA-NOSE could identify VOC patterns indicative of *Brucella* exposure with excellent discriminative power, using a statistical algorithm. The patterns were not affected by the animals' environment and the discriminative power of the approach was stable over time.

### **Publications:**

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*. Analytical Chemistry. *In press*.

**Polar bears as a sentinel for emerging wildlife zoonoses with implications for public health in Alaska: past and present occurrence of *Brucella* spp., *Coxiella burnetii*, and *Toxoplasma gondii* in southern Beaufort Sea polar bears**

**WiLDIT Staff:** Jack Rhyan Pauline Nol, Matt McCollum

**Collaborating Agencies:** USGS/Alaska Science Center, Colorado State University, USDA/APHIS/Vs/CEAH, USDA/APHIS/Vs/NVSL

**Background:** Polar bears are an apex species in the Arctic. Although primarily a marine predator, their occurrence on land is increasing. Because of this, and the resultant scavenging, polar bears may now be exposed to an increasing variety of diseases. The use of polar bears as sentinels to zoonotic disease can provide vital insight into the Arctic ecosystem and the health of other wildlife species and humans. *Brucella* spp., *Coxiella burnetii*, and *Toxoplasma gondii* are contagious, zoonotic disease agents of regulatory, economic, and public health importance which have been identified in humans and wildlife. These diseases have implications on human and animal health and food safety, particularly in the Arctic where humans rely on a variety of wildlife species for subsistence. Environmental changes such as reductions in sea ice coverage, along with changes in bear behavior (*i.e.*, increased time spent on land, nutritional stress, increased density at bowhead whale remain piles), and increased human activity in the Arctic provide greater opportunities for intra- and inter-species interactions and zoonotic disease transmission.

**Study Design:** This study will analyze archived samples and collect prospective samples from the southern Beaufort Sea polar bear population in conjunction with the U.S. Geological Survey's Polar Bear Research Program. The objectives of this project are to determine the current and past prevalence of *Brucella* spp., *C. burnetii*, and *T. gondii* among polar bears, identify potential co-infections with these organisms, investigate the source(s) of these disease agents in polar bears, and evaluate risk factors for exposure

**Results and Conclusions:** Pending

**Publications:** Pending

## **Delphi survey defining Polar bear health**

**Staff:** Pauline Nol

**Collaborating Agencies:** USGS/Alaska Science Center, Colorado State University, USDA/APHIS/VS/CEAH

**Background:** The topic of health among wildlife populations has received increasing attention as an important concept in management and policy responses, yet ‘health’ remains a difficult concept to define and assess. Vongraven et al. (2012) noted the importance of monitoring the health of polar bears and included it as a topical area in their circumpolar monitoring framework. This project seeks to build upon that effort and derive an expert-based, wholistic definition of polar bear health so that it, and the population-level effects, can be meaningfully studied.

**Study Design:** This research project is designed to outline a working definition of ‘health’ as it applies to polar bears and to consider how it may influence research and management decisions. A series of two surveys will be distributed to selected polar bear experts (n=10) as part of a Delphi exercise to explore the concept of polar bear health. The goals of the exercise are to define polar bear health, identify current concerns regarding polar bear health, and outline important indicators to monitor health in polar bears. The work is intended to serve as a guide in advance of the 2015 Polar Bear Specialist Group meeting to unify monitoring plans among the polar bear regions, including health plans.

**Results and Conclusions:** Pending

**Publications:** Pending

## **Infectious agents in polar bears and associated health effects: A systematic review of the literature**

**WiLDIT Staff:** Pauline Nol

**Collaborating Agencies:** Colorado State University, USDA/APHIS/VS/SPRS/CEAH

**Background:** In recent years, there has been increased awareness of the role of ecologic change on the health of individuals and populations. Climate change is a significant driver of ecologic change and effects of climate change are expected to be most severe in polar regions and are anticipated to have health impacts on marine mammals. The polar bear was listed as threatened May 2008 and projections for this species indicate severe population declines by the end of the 21<sup>st</sup> century. In light of these predictions, in order to effectively monitor polar bear health, it is necessary to understand both existing and potential pathogens that could impact polar bear populations. The objective of this study is to systematically review scientific literature on infectious agents in polar bears to identify important issues or knowledge gaps that will assist in the development of management and research strategies in the future.

**Study Design:** Databases used included PubMed, Web of Science, Science Direct, and Zoological Record Plus. Text word searches were conducted related to polar bear health and ecology (infect\*, illness\*, mortality, lesion\*, transmiss\*, disease\*, & pathol\*) and infectious agents in polar bears divided by type of agent. Infectious disease search parameters were run for bacteria (bacteria\*, brucella\*), viruses (virus, viral), fungi (fung\*, myco\*), and parasites (parasit\*). Each of the aforementioned search terms was run in addition to the phrase "*polar bear*" AND. The literature search was conducted between June 2013 and October 2013.

**Results and Conclusions:** To date- the literature search was conducted between June 2013 and October 2013, and 676 papers were identified for initial review. Papers in languages other than English were excluded (n=6). Additional results and conclusions pending.

**Publications:** Pending

## **Immune response of bison to *Brucella*-specific lipids**

**WiLDIT Staff:** Jack Rhyan, Pauline Nol, Matt McCollum

**Collaborating Agencies:** Colorado State University, USDA/APHIS/VS/NVSL, USDA/ARS/NADC

**Background:** *Brucella* species are select agents and the cause of chronic disease and abortion in animals, and a severe chronic disease in humans. Although once considered to be eradicated in the US, brucellosis persists in wildlife populations, particularly in the Greater Yellowstone area, and is now considered an emerging disease in cattle in that area. Currently available diagnostic tests have poor specificity, and culturing the organism, the diagnostic gold standard, is difficult. In order to contain and/or fully eradicate this disease, more specific diagnostic tests are needed. Recently, CSU collaborators identified seven polar *Brucella* lipids that are specific for *B. melitensis*, *B. abortus* and *B. suis*. Furthermore, three of the lipids provide strong serological activity with sera from bison with brucellosis without cross-reactivity to lipids from *Yersinia enterocolitica* O:9. Two sero-dominant lipids have been characterized: lipid-A (m/z 693.61 Da) and lipid-B (m/z 814.63 Da). The chemical structure of lipid-A already determined as an ornithine lipid. This project's aims are to determine the chemical structure of an immunodominant *Brucella* lipid (lipid-B) and study the immune responses to lipid-B and the ornithine lipid in naturally-infected bison.

**Study Design:** Calves born to *Brucella*-positive cows and *Brucella*-negative cows, that are part of the WiLDIT immunocontraception study in Montana, will be sampled over three years to monitor cellular and humoral immune responses to lipid-B and the ornithine lipid.

**Results and Conclusions:** Pending

**Publications:** Pending

## **A benefit-cost analysis decision framework for disease mitigation at the wildlife-livestock interface.**

**WiLDIT Staff:** P. Nol, J. Rhyan

**Collaborating Agencies:** USDA/APHIS/WS/ NWRC, USDA/APHIS/VS/CEAH

**Background:** The economics of managing diseases at the wildlife-livestock interface have received heightened attention as agricultural and natural resource agencies struggle to tackle growing risks to animal health. In the fiscal landscape of increased scrutiny and shrinking budgets, resource managers seek to maximize the benefits and minimize the costs of disease mitigation efforts. To address this issue, a benefit-cost analysis decision model was developed to help users make informed choices about whether and how to target disease management efforts in wildlife and livestock populations.

**Outcome:** A benefit-cost analysis model was designed to measure and compare the value of management actions in terms of the direct benefits to impacted sectors (livestock, wildlife, humans and their companion animals) and costs resulting from the management actions chosen to mitigate disease transmission in wildlife and livestock populations.

**Publications:** Shwiff, S. A., S. J. Sweeney, R. S. Miller, M. L. Farnsworth, **P. Nol**, S. S. Shwiff, and A. M. Anderson. A benefit-cost analysis decision framework for disease mitigation at the wildlife-livestock interface. *Submitted*.

## **Immunogenicity and Efficacy of Oral and Parenteral Rough *Brucella suis* Vaccine Delivered In Domestic and Feral Swine**

**WiLDIT Staff:** P. Nol, J, Rhyan

**Collaborating Agencies:** USDA/ARS/NADC, USDA/APHIS/WS/ NWRC, Virginia Polytechnic Institute and State University

**Background:** Feral swine cause extensive damage to natural and agricultural resources and also serve as reservoirs for infectious diseases such as swine brucellosis, caused by *Brucella suis*. Not only does this disease have the potential to spill over into domestic swine populations when feral swine come into contact with outdoor pig operations, swine brucellosis also serves as a public health threat to hunters and other individuals who process infected feral pig carcasses. An oral vaccine protecting feral swine from brucellosis would serve as an important management tool to reduce disease in those populations.

**Study Design:** The objective of the current study was to evaluate the safety and efficacy of oral or parenteral vaccination of domestic or feral swine with *Brucella suis* strain 353-1 (353-1). Domestic male swine were randomly assigned to control (n=8) or oral or parenteral vaccination treatments (n=12/trt) which received  $1.9 \times 10^{10}$  CFU of strain 353-1 in 2 ml of saline. In a similar manner, feral swine of Texas stock were randomly assigned to equivalent control (n=10), parenteral (n=9), or oral (n=9) vaccination treatments. Clearance and tissue distribution were determined by obtaining samples for microbiologic evaluation at necropsy at 4 and 8 wks post-inoculation. Vaccine efficacy was determined by conjunctival challenge with  $5 \times 10^7$  CFU of *B. suis* strain 3B at 17 weeks after inoculation and microbiologic evaluation of tissues obtained at necropsy at 4 or 5 weeks post-challenge.

**Results and Conclusions:** Parenteral or oral vaccination induced humoral and peripheral blood mononuclear proliferative responses that were greater than responses of control animals. Non-vaccinated feral swine had greater tissue colonization after challenge than domestic swine. Both oral and parenteral vaccination with 353-1 vaccine induced significant reductions in tissue colonization after experimental challenge with strain 3B. The data suggest strain 353-1 is an efficacious vaccine for use in preventing brucellosis in both domestic and feral swine.

**Publications:** In preparation



## **Determination of tissue persistence of *Mycobacterium bovis* BCG in Texas-origin feral swine orally vaccinated with *Mycobacterium bovis* BCG**

**WiLDIT Staff:** Pauline Nol, Jack Rhyan, Matt McCollum

**Background:** Bovine tuberculosis (bTB) is present in feral swine populations on Molokai, Hawaii, USA (1). *Mycobacterium bovis* BCG (BCG), an attenuated vaccine strain of *M. bovis*, is being considered for use on Molokai to manage bTB in its feral swine population. Since BCG is a live bacterial vaccine and feral swine on Molokai are hunted year-round, it is important to know if and how long BCG persists in various tissues with the potential for transmission to humans and other hosts.

**Collaborators:** Colorado State University, USDA/APHIS/VS/NVSL, IREC Spain

**Study Design:** 16 feral swine were hand fed an oral bait formulated for European wild boar incorporating a plastic vial containing  $1 \times 10^6$  cfu BCG. Four feral swine were each given bait without BCG. At 1, 3, 6, and 9 mos post-vaccination (PV), tissues were collected from 4 vaccinated animals. At 9 mos PV tissues were collected from controls. Tissue pools for *Mycobacterium* spp. culture: head pool, thoracic pool, abdominal pool, and muscle

**Results and Conclusions:** All but one of the vaccinates consumed a bait with vaccine vial. One animal (unknown) consumed the bait but not vaccine vial. 14.5% tissues were contaminated and could not be cultured. None of the remaining tissues grew any species of *Mycobacterium*.

**Publications:** In Preparation

## **The potential for transmission of BCG from orally vaccinated white-tailed deer (*Odocoileus virginianus*) to cattle (*Bos taurus*) through a contaminated environment**

**WiLDIT Staff:** P. Nol, J. Rhyan, M. McCollum, Karl Held

**Collaborating Agencies:** USDA/APHIS/VS/NVSL; Colorado State University

**Background:** White-tailed deer experimentally infected with a virulent strain of *Mycobacterium bovis* have been shown to transmit the bacterium to other deer and cattle by sharing of pen waste and feed. There was therefore a need to understand the risk of transmission of *M. bovis* bacille Calmette-Guerin (BCG) vaccine from orally vaccinated white-tailed deer to other deer and cattle.

**Study Design:** Fourteen white-tailed deer were orally vaccinated with  $1 \times 10^9$  colony forming units BCG in lipid-formulated baits and housed them with nine non-vaccinated deer. Each day we exposed the same seven naïve cattle to pen space utilized by the deer. Before vaccination and every 60 days until the end of the study, we performed tuberculin skin testing on deer and cattle, as well as interferon-gamma testing in cattle, to detect cellular immune response to BCG exposure. At approximately 27 weeks all cattle and deer were euthanized and necropsied.

**Results and Conclusions:** None of the cattle converted on either caudal fold, comparative cervical tests, or interferon-gamma assay. None of the cattle were culture positive for BCG. Although there was immunological evidence that BCG transmission occurred from deer to deer, we were unable to detect immunological or microbiological evidence of transmission to cattle. This study suggests that the risk is likely to be low that BCG-vaccinated white-tailed deer would cause domestic cattle to react to the tuberculin skin test or interferon-gamma test through exposure to a BCG-contaminated environment.

### **Publications:**

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.

## **Feasibility of Quarantine Procedures of bison calves from Yellowstone National Park for conservation of brucellosis free bison.**

**WiLDIT Staff:** Jack Rhyan, Matt McCollum, Pauline Nol, Karl Held

**Collaborating Agencies:** Montana Department of Fish, Wildlife and Parks, MT Dept. of Livestock, DOI/National Park Service/Yellowstone National Park, USDA/APHIS/VS/NVSL

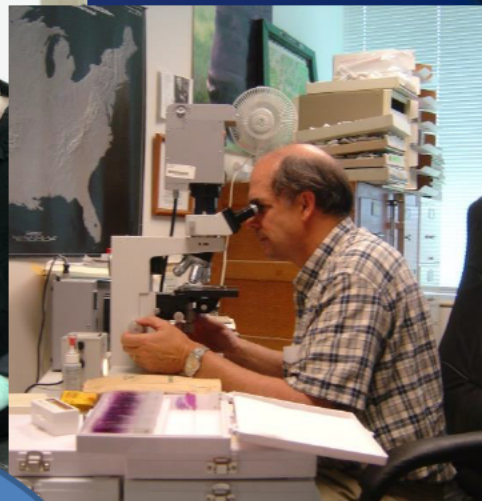
**Background:** In the Record of Decision of the Interagency Bison Management Plan signed in 2000, the agencies were directed to investigate the feasibility of quarantine. In 1997, Congress set aside one million dollars for the construction of a bison quarantine facility. Environmental Assessments for the BQFS were conducted for Phases 1, 2, and 3.

**Study Design:** Two cohorts of 100 seronegative calves (2005 and 2008) were collected from a bison capture effort as part of the routine IBMP management actions. The calves were tested monthly until all were seronegative two months consecutively. Half the calves were then sent to slaughter and specimens collected for *Brucella* culture. Bison were repeatedly tested and maintained through first pregnancy. Pregnant cows were blood tested at least twice per year and cow/calf pairs were sampled immediately post calving for evidence of *B. abortus* infection. One or more blood samples were collected from all animals at 6 months post calving and beyond. Graduating bison were required to be seronegative and culture negative and their entire enclosure cohort was also required to be seronegative and culture negative throughout the study. Graduating bison were able to be relocated to tribal or public lands. They must remain captive for five years during which they will be monitored by serology yearly.

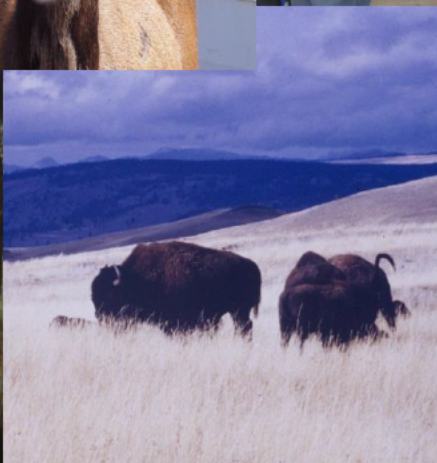
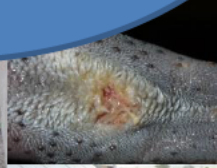
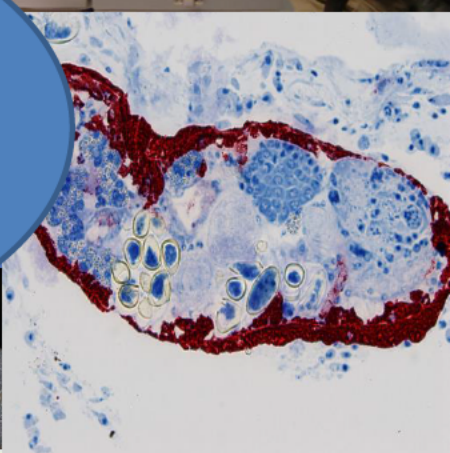
**Results and Conclusions:** After testing negative for two months consecutively in the first year, none of the bison seroconverted for the duration of the study. No calves born to any of the bred females tested positive for brucellosis, no *B. abortus* was cultured from any of the dams after parturition, and none of the cows or calves displayed a titer for *B. abortus* antibodies. No *B. abortus* was cultured from any of the sacrificed sero-negative animals. Both cohorts have been successfully relocated. They are both in the routine yearly monitoring phase.

**Publications:** Frey, R. K., 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*.





# Future Projects





**Natural transmission of *Brucella abortus* from naturally-infected elk to naïve elk in a paddock; demonstration of a natural transmission challenge potentially useful for vaccine testing**

**WiLDIT Staff:** Jack Rhyan, Pauline Nol, Matt McCollum, Karl Held

**Collaborating Agencies:** WY GFC, USDA/APHIS/VS/NVSL

**Background:** The need to develop an effective brucellosis vaccine in elk is high given the increase in seroprevalence in GYA elk herds. To date no vaccine has been found to be over 30% effective in protecting elk from *B. abortus*-induced abortion. In addition, the current experimental infection model in elk is inconsistent and cost prohibitive. A reliable and realistic model of *Brucella* infection in elk must be developed in order to effectively test vaccines in this species.

**Study Design:** In winter 2013-2014, between 12 and 20 seropositive, pregnant, young, female elk will be transported to the Colorado State University-APHIS wildlife research facility in Fort Collins, CO. Elk will be placed with approximately 20 seronegative elk obtained from *Brucella*-free commercial sources. Seropositive and negative elk will be comingled in a paddock. If abortions occur, fetuses will be left in place for 24 hours and remotely monitored by motion-activated video cameras. Afterward, fetuses will be necropsied, cultured and incinerated. After seropositive elk have calved, all elk will be bled for serology and blood culture.

**Results and Conclusions:** Pending

**Publications:** Pending

## **Evaluation of volatile organic compounds (VOCs) from naturally infected bison as a screening tool to detect *Brucella abortus* infection**

**WiLDIT Staff:** Jack Rhyan, Pauline Nol, Matt McCollum

**Collaborating Agencies:** Technion, Israel, USDA/APHIS/WS/ NWRC, USDA/APHIS/VS/SPRS, National Park Service

**Background:** An emerging approach for diagnosing infectious disease at its earliest stages relies on volatile organic compounds (VOCs) that are emitted from the infectious agent and/or the host. This study is a continuation of previous studies and will further explore the utility of breath testing for the detection of *B. abortus* infection in bison. In pilot studies, different patterns of VOCs have been detected in breath of *Mycobacterium bovis*-infected cattle compared to uninfected animals. Also feces from *M. bovis*-infected deer could be differentiated from uninfected deer by VOC analysis. An additional study showed that *Brucella abortus*-seropositive bison could be distinguished from seronegative animals by the patterns of VOCs in breath. The purpose of this study is to further evaluate VOC analysis as a screening tool for *B. abortus*-infected bison.

**Study Design:** Breath and fecal samples will be collected from 60 adult bison captured at the Stevens Creek and/or Duck Creek traps in winter 2104. Following specimen collection, serology results on blood samples routinely collected at the traps will be awaited. If animals are sent to slaughter, bacteriologic results will be awaited. The goal is to identify at least 15 animals that are seropositive and culture positive to be compared to at least 15 animals that are seronegative, and ideally culture negative. If seronegative animals are not sent to slaughter, then negative status will be based solely on serostatus. Breath and fecal specimens will then be divided into 3 groups: at least 10 positives to serve as positive controls, at least 10 negatives to serve as negative controls, and a group made up of at least 5 positives and 5 negatives to serve as “unknowns.” Specimens will then be tested at two laboratories by gas chromatography-mass spectrophotometry (GC-MS) and an electronic nose as in past studies. If known positive specimens can be distinguished from known negative specimens, “unknown” specimens will then be tested and classified.

**Results and Conclusions:** Pending

**Publications:** Pending

## **Inactivated *Mycobacterium bovis* vaccine in feral swine**

**WiLDIT Staff:** Pauline Nol, Jack Rhyan, Matt McCollum

**Background:** Researchers at IREC, Ciudad Real, Spain have tested an inactivated *M. bovis* strain for efficacy as a vaccine in wild boar against disease caused by *M. bovis* infection. Administered orally, the vaccine is very comparable in performance to BCG in wild boar. We propose a study to test efficacy of this vaccine in Hawaiian feral swine. A killed vaccine, as opposed to live BCG, will be much easier to administer in a field situation, since it is environmentally safe and more stable under various conditions. The target population in the US for this vaccine would be the *M. bovis*-infected feral swine population on Molokai Island in Hawaii.

**Collaborators:** Colorado State University, USDA/APHIS/VS/NVSL, USDA/APHIS/WS/NWRC, IREC Spain

### **Study Design:**

Thirty-six three to four month old feral swine piglets of Hawaiian origin will be used for this project and will initially be housed at the APHI Wildlife Research Facility. They will be broken into three treatment groups: 1) oral BCG (n=12); 2) oral heat inactivated *M. bovis* (n=12); 3) oral PBS (controls: n=12). Vaccination will consist of offering pigs bait containing  $10^6$  cfu BCG or the equivalent of  $10^6$  cfu inactivated vaccine. Control pigs will be fed bait containing same volume PBS. Pigs will be held for 2 months after vaccination and then moved to a large animal BL3 facility at CSU. They will be allowed to acclimate for two weeks. They will be challenged with  $10^4$  cfu Molokai field strain *M. bovis* via the oropharyngeal route. The inoculum will be administered via syringe and catheter to the oropharynx. Humoral and cellular immune responses will be monitored throughout the study. Four months after challenge, animals will be euthanized and necropsied for tissue culture and histopathology.

Results and Conclusions: Pending

Publications: Pending



## **A benefit-cost analysis decision framework for brucellosis management in the Greater Yellowstone Area.**

**WiLDIT Staff:** Jack Rhyan Pauline Nol, Matt McCollum

**Collaborating Agencies:** USDA/APHIS/WS/ NWRC, USDA/APHIS/VS/STAS/CEAH, USDA/APHIS/VS/SPRS

**Background:** Brucellosis in the GYA has not been eradicated from wild bison populations, but remains contained through costly efforts by the GYA states and federal government agencies. Brucellosis in elk herds is increasing in prevalence and entering new ranges. Determining costs and benefits of managing wild elk and bison herds (vaccination, culling, hazing, etc.) in addition to managing ranched bison and cattle herds would be very beneficial to determine the feasibility of wildlife disease management in the GYA in the long term. A benefit-cost analysis model (Shwiff et al., submitted) was designed to measure and compare the value of management actions in terms of the direct benefits to impacted sectors (livestock, wildlife, humans and their companion animals) and costs resulting from the management actions chosen to mitigate disease transmission in wildlife and livestock populations.

Outcome: Pending

Publications: Pending

## **Efficacy of RB51 vaccination with booster in free-ranging Yellowstone bison.**

**WiLDIT Staff:** Jack Rhyan Pauline Nol, Matt McCollum

**Collaborating Agencies:** USDA/APHIS/VS/NVSL, USDA/APHIS/VS/SPRS, National Park Service

**Background:** *Brucella abortus* vaccine strain RB51 when given parenterally and then boosted after one year has shown good efficacy in protecting bison against experimental challenge with *B. abortus* under BSL-3 conditions (Olsen et al., unpublished data). Our objectives are to 1) determine if boosted vaccination delivered remotely or by hand decreases shedding of *B. abortus*; 2) Determine if boosted vaccination by either technique reduces *B. abortus* abortions; 3) Determine if boosted vaccination by either technique affects antibody response to *B. abortus*.

### **Study Design (Proposed):**

**Fall 2014:** 75 radiocollared animals will be captured by tranquilizer dart or helicopter net gun. Serologic status will be determined by FPA and/or card test.

Animals will be placed in one of 3 groups:

1. Nonvaccinated controls (n=25-32)
2. RB51 hand vaccinated treatments (n=25-32)
3. RB51 remotely vaccinated treatments (n=25-32)

Remote vaccination will be accomplished by biobullet or dart fired at a distance of 25 feet. A target sample size is 25-32 bison in each group. If this sample is not obtained in the Spring of 2014, the remainder will be added in Fall 2014 or Spring 2015. If bison identified as seronegative in Spring 2014 have seroconverted, they will be replaced with other radiocollared animals from the 25 animal reserve group. Any bison that seroconvert following the first vaccination will remain in the study. Blood specimens for serology and culture will be obtained from any immobilized bison throughout the study.

**January-February 2015:** Bison that started the study as yearlings in Spring 2014 (turning 2 in May 2014) will be captured by tranquilizer dart or helicopter net gun and pregnancy tested. Pregnant animals will have vaginal transmitters installed.

**Spring 2015:** Any study bison caught at the traps will be examined and blood specimens collected. If bison are still needed to fill the target sample size of the groups, those will be radiocollared and begin the study.

**Post calving 2015:** In the first 5 days post calving, bison cows will be immobilized and calves captured when possible. Specimens collected will include blood, vaginal swab, uterine swab, and milk. Calving sites will be examined and samples of placenta or aborted fetuses collected for culture. Colony counts on milk and blood will be done.

Uterine and vaginal swabs will be cultured and colony formation observed. Plates will be rated as negative, or 1+ to 3+.

**Fall 2015:** Animals in the remote vaccination group will be boosted by remote vaccination. Animals in the hand vaccinated group will be captured and boosted by hand vaccination.

**January-February 2016:** Two-year-old and older bison will be captured and pregnancy tested. Pregnant animals will have vaginal transmitters installed.

**Post calving 2016:** same as 2015

**Results and Conclusions:** Pending

**Publications:** Pending

## WiLDIT Publications (last 7 years)

Olsen, S. C., J. Wilson-Welder, **P. Nol**, **J. Rhyan**, N. Srirangathan. Immunogenicity and efficacy of an oral rough *Brucella suis* vaccine delivered in domestic and feral swine. *In prep.*

**Rhyan, J.**, **M. McCollum**, T Gidlewski, M. Shalev, G. Ward, B. Donahue, J. Arzt, F. Mohamed, **P. Nol**, M. Ding, S. Metwally, T. McKenna, M. Salman. Foot-and-mouth disease in pronghorn (*Antilocapra americana*) susceptibility, Intra-and interspecies transmission, clinical signs, lesions and laboratory results. *In prep.*

**McCollum, M.**, **J. Rhyan**, **P. Nol**, S. Hennager, and M. Salman Comparison of serological techniques in bighorn sheep experimentally infected with *Brucella ovis*. *In prep.*

**Nol, P.**, S. Robbe-Austerman, **J. Rhyan**, **M. McCollum**, J. Triantis, B. Beltrán-Beck, and M. Salman.

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# Wildlife/Livestock Disease Investigations Team Team (WiLDIT)

USDA APHIS Veterinary Services

“Developing science-based solutions to disease problems at the wildlife/domestic animal interface”

Jack Rhyan  
Matt McCollum  
Karl Held  
Pauline Nol





# WiLDIT Activity Areas

- Developmental work (collaborating with ARS, NWRC, YNP, CSU, others)
- Diagnostics/surveillance
- Consultation/liaison
- Training



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# WiLDIT Diseases Focus

- Brucellosis in Greater Yellowstone Area wildlife
- Brucellosis in feral swine
- TB in deer and feral swine
- CWD in cervids
- FMD threat in North American wildlife



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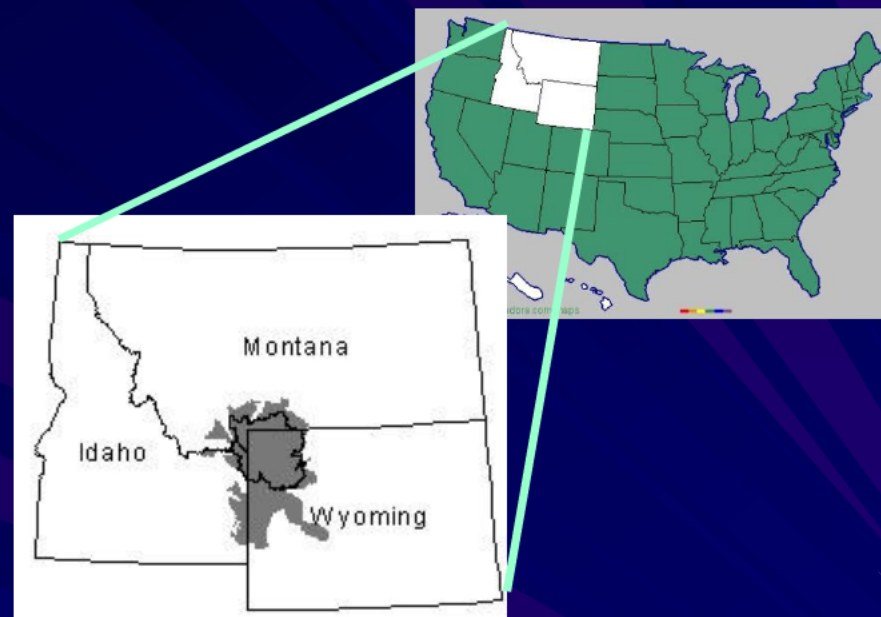




# Brucellosis in Greater Yellowstone Area Elk and Bison



- Introduced early 1900's
- Bison: 50-80% seropositive
- Elk: 3.7-40% seropositive
- Bison: *B. abortus* biovars 1 and 2
- Elk: *B. abortus* biovars 1 and 4
- Elk implicated in most cattle herd cases



# Brucellosis in Wildlife



# Brucellosis in Greater Yellowstone Area Elk and Bison

## Yellowstone Bison Quarantine Feasibility Study

- Determine feasibility, efficacy, and associated risks of using the USDA's Uniform Methods & Rules to Eradicate Brucellosis protocol to qualify bison from Yellowstone National Park as free of brucellosis
- 2005-2012: Graduated 148 adult bison from quarantine





# WiLDIT-Brucellosis in the GYA

Develop nonlethal strategies to eradicate brucellosis from GYA bison and elk

## ■ Bison

- Contraception
- Sustained release antibiotics
- Therapeutic vaccination

## ■ Elk

- Vaccine
- Contraception



# WiLDIT Bison Contraception Studies

- Does use of contraceptive vaccine decrease shedding of *B. abortus*? Montana-44 bison
- Duration of infertility southern Colorado - 20 bison



# WiLDIT-Brucellosis

- Elk/bison/cattle: ecology of disease
- Bison: venereal transmission
- Feral swine: oral vaccination for *B. suis*
- Diagnostics
  - Bison: detection of brucellosis by breath analysis for VOCs
  - Multispecies: lipid antigens uniquely produced by individual *Brucella* spp.



# WiLDIT Bovine TB Work

- Development of oral TB vaccines:
  - Michigan deer
  - Molokai feral pigs
- Detection of VOCs for the screening/diagnosis of TB in wildlife and livestock



001319



# FMD Susceptibility & Transmission in North American Wildlife

- Experimental infections in bison, elk, pronghorn, & mule deer.





# Current/Future Work – Wildlife Disease Surveillance and Control

## ■ Development and demonstration of techniques for wildlife disease surveillance

- Use of drones for census and disease detection
- Thermal imaging to conduct census and detect febrile animals



# Current/Future Work – Feral Swine

- Techniques for feral swine (develop and prove 4 point program using temporary feeding sites)
  - Implement feeding sites to keep feral swine from leaving area
  - Motion-triggered infrared imaging at feeder site for disease monitoring (fever or lesions)
  - Detect disease-specific VOCs
  - Using pig-specific feeder: kill, contraceptive or vaccinate (*B. suis*, TB, swine flu, CSF, FMD, etc.)





# APHI/APHIS Wildlife Research Facility



- On CSU land south of NWRC – shared between APHIS and CSU-APHI
- Currently housing bison and feral pigs



Wildlife Livestock Disease Investigations Team  
Project Funding Update 2017-2019  
23 January 2017

**1. Development of GonaCon, an immunocontraceptive vaccine, as a method to stop *Brucella abortus* transmission in bison and elk.**

**Funding source:** **Cattle Health \$108,000**

**Current Projects: \$108,000**

1) Great Sand Dunes Evaluation of GonaCon as an immunocontraceptive in bison-complete November 2017

Travel \$500

2) Montana Corwin Springs Study to be completed 2019

Travel: \$1000/year for three years

Feed, housing, health maintenance for animals moved to Fort Collins

\$35,000/year for three years

**2. Development of DryDart, a vehicle to deliver lyophilized or powdered, pelleted medicaments to animals.**

**Funding source:** **Cattle Health \$21,000**

**Current Projects:** Completed

**Future Projects:**

1) NADC collaborative study-Evaluation of Dry Dart RB51 lyophilized payload in 10 bison with experimental *B. abortus* challenge

Animals and travel: \$21,000

2) Field Trial-Vaccination of Yellowstone bison with RB51 \$??

**3. Development of oral fluid collection studies for feral swine.**

**Funding source:** **National Feral Swine Initiative \$80,900**

**Current Projects:**

1) Texas A and M pens studies (capture and collection from pigs naturally infected with *B. suis* or pseudorabies virus)

Cooperative Agreement 2017 \$11,000

Wildlife Livestock Disease Investigations Team  
Project Funding Update 2017-2019  
23 January 2017

Cooperative Agreement 2018	\$11,300
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Cooperative Agreement 2019	\$11,600
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\$33,900 total through 2019

2) Molecular evaluation of collection devices for *M. bovis*, Colorado State University

Cooperative Agreement 2017	\$14,100
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Cooperative Agreement 2018	\$14,500
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Cooperative Agreement 2019	\$14,900
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\$43,500 total through 2019

3) Evaluation of acceptance of oral fluids collection devices by free-ranging feral swine, University of Florida and University of Georgia. 2017 study

Travel	\$2,500
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Supplies	\$1,000
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**4. Development of *Brucella abortus* natural infection model in elk**

Funding source:	<u><b>Cattle Health \$210,500</b></u>
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**Current Projects:**

1) *Brucella abortus* natural infection model in elk

Feed:	\$67,500/year through 2019
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Travel, health, drugs, misc.:	\$8,000 for three years
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**5. Evaluation of killed preparations of *Brucella abortus* and *Mycobacterium bovis* in mice, elk, and feral swine.**

Funding source:	<u><b>Cattle Health \$142,000</b></u>
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**National Feral Swine Initiative \$157,700**

**Current Projects:**

1) Efficacy of mucosally-administered lyophilized killed *B. abortus* in protecting mice against mucosal challenge by virulent *B. abortus*. to be completed spring 2017

Wildlife Livestock Disease Investigations Team  
Project Funding Update 2017-2019  
23 January 2017

2016 Cooperative Agreement with Colorado State University in place

\$19,777

2) Efficacy of mucosally-administered killed *M. bovis* in protecting feral swine of Molokai origin against mucosal challenge by virulent *M. bovis*. To be completed summer 2017

2016 Cooperative Agreement with Colorado State University in place

\$82,877

WiLDIT swine feed and husbandry \$2,500

**Future Projects:**

1) Evaluation of mucosally-administered lyophilized killed *B. abortus* in protecting elk against natural challenge by virulent *B. abortus*.

Projected minimum of two trials over two years

Feed \$67,500/year for two years

Drugs, health, misc. \$3,500/year for two years

2) Evaluation of mucosally-administered lyophilized killed *M. bovis* in protecting feral swine against natural challenge by virulent *M. bovis*.

Cooperative Agreements with Colorado State University

Cooperative Agreement 2017 \$50,000

Cooperative Agreement 2018 \$50,900

Cooperative Agreement 2019 \$51,800

Total \$152,700 total through 2019

Feed and husbandry for three years \$5,000

**6. Development and evaluation of remote disease detection tools using volatile organic compounds**

**Funding source:** **National Feral Swine Initiative \$115,800**

**Current Project:** 2016 collection and analysis of breath and fecal samples from *M. bovis*-infected Spanish wild boar and feral swine



Wildlife Livestock Disease Investigations Team  
Project Funding Update 2017-2019  
23 January 2017

1) 2016 Cooperative Agreement in place with Rovira i Virgili University, Tarragona, Spain. Work is being completed.

**Future Projects:**

1) Cooperative Agreements with Rovira i Virgili University, Tarragona, Spain

Cooperative Agreement 2017	\$29,000
Cooperative Agreement 2018	\$29,500
Cooperative Agreement 2019	\$30,100

Total \$88,600 through 2019

2) Cooperative Agreements with IREC, University Castilla La Mancha, Ciudad Real, Spain

Cooperative Agreement 2017	\$13,500
Cooperative Agreement 2018	\$13,700

Total \$27,200 through 2018

**7. Development of Safe and Effective Immobilization Protocols for Wild Swine**

**Funding source:** **National Feral Swine Initiative \$5,000**

**Future Project in 2017:** Evaluate three different combinations of immobilization drugs in feral swine for safety, efficacy, and reversibility in a field setting-joint project with WS

Feed and drugs	\$5,000
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**8. Use of Assisted Reproductive Techniques to Produce Brucellosis-free Bison with Yellowstone Genetics**

**Funding source** **Cattle Health \$2,000**

Drugs, health, support	\$2,000
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## **1. Development of GonaCon, an immunocontraceptive vaccine, as a method to stop *Brucella abortus* transmission in bison and elk**

**Collaborations:** APHIS/VS/SPRS, USDA, ARS

**Description of objective or deliverable:** The deliverable for this series of experiments is the demonstration and verification previous studies we have found GonaCon to be an effective means of producing infertility in the majority of bison and elk. In current studies, we are measuring the duration of infertility following one vaccination, and are demonstrating the efficacy of the vaccine in eliminating or markedly reducing *B. abortus* shedding in bison. Future studies should include a field trial in bison and, potentially, elk.

**Benefit to VS Program:** The National Park Service has objected to any test-and-slaughter approach in bison. WY Game and Fish demonstrated a test-and-cull approach would markedly reduce prevalence in elk but is reluctant to pursue it because of cost and public resistance. The development of a non-lethal means of decreasing transmission of brucellosis should provide a valuable disease management tool for use by agencies.

**Time Sensitivity:** Currently, between 2 and 4 herds of cattle and ranched bison in the GYA are infected with brucellosis transmitted from elk, each year. There are few tools to mitigate this spread of the disease. These studies have been ongoing for 2 years and need to continue to completion.

## **2. Development of DryDart, a vehicle to deliver lyophilized or powdered, pelleted medicaments to animals**

**Description of objective or deliverable:** The deliverable for this series of small studies is a product that will effectively remotely deliver pelleted medicaments (designed primarily with RB51 in mind) to animals. Developmental work has produced an effective prototype of the DryDart and shown it to be effective in delivering RB51 to bison. Vaccinated bison developed titers to RB51 at the same rate as hand vaccinated animals. Future work will improve range and accuracy and will evaluate its usefulness in delivering immobilizing agents.

**Benefit to VS Program:** The development of DryDart technology will replace Biobullet technology which was used for years in vaccinating elk against brucellosis. Biobullets are no longer produced. If accepted by Yellowstone National Park authorities, DryDart would provide an excellent method of remotely delivering brucellosis vaccine to bison and/or elk.

**Time Sensitivity:** Currently, between 2 and 4 herds of cattle and ranched bison in the GYA are infected with brucellosis transmitted from elk, each year. There are few tools to mitigate this spread of the disease. These studies have been ongoing for 2 years and need to continue to completion.

### 3. Development of oral fluid collection studies for feral swine

**Collaborations:** APHIS/Wildlife Services

**Description of objective or deliverable:** The deliverable for this series of studies is the development and testing of a practical and effective means for sampling the presence/absence of disease exposure or occurrence in feral swine populations. We are experimenting with collection of swine oral fluids using methods similar to those used in commercial swine operations (cotton rope lengths) but adapted for wild feral swine populations. Once collected fluids would be analyzed using standard PCR and ELISA techniques to detect for pathogens and/or antibodies. Studies conducted on commercial swine have been successful at detecting porcine reproductive and respiratory syndrome virus (PRRSV) and antibodies as well as other pathogens including *E. coli*, African swine fever and Classical swine fever.

One component of this series of experiments will be pen side collection of oral fluids on captured feral swine housed in facilities at Texas A&M Kingsville. Swine housed there will be captured from Texas free ranging populations where *Brucella suis* and Pseudorabies (PRV) are known to be present. Trap side serum based tests will identify both positive and negative individuals for the above referenced pathogens and both types of individuals will be transferred to the pen facility. Once housed ropes will be placed in pens and subsequently collected to extract oral fluid for testing. We will also allow a subset of the ropes to air dry and determine if oral fluids that have dried and then are washed from the rope still produce identifiable pathogens or antibodies. We will further this line of investigations by expanding our dried rope work to include environmental chamber experiments where we can determine sample stability when subjected to various temperature and moisture gradients.

A second component will determine if oral fluids collected from captive feral swine vaccinated with BCG or killed *M. bovis* show antibody response to vaccination or evidence of oral shedding (BCG vaccinates). In addition, we will determine presence of antibody response and/or oral *M. bovis* shedding after challenge with virulent *M. bovis*. Baseline and repeated post-vaccination and post-infection fluids will be compared to serum sample values as well as Volatile Organic Compounds (VOCs) collected as part of the TB vaccine challenge study.

**Benefit to VS program:** Oral fluid diagnostics for infectious and non-infectious diseases are a functionally and economically effective method of collecting and analyzing a large volume of epidemiological samples. Oral fluid sampling has been used extensively in human medicine (e.g. HIV, measles) but is less frequently used in veterinary medicine. Additionally most veterinary usages occur either in commercial livestock or domestic pet facilities. The technique is relatively novel in the field of wildlife ecology due to potential challenges with the timely collection of the rope device and pathogen detectability. Our studies will hopefully demonstrate the efficacy of the approach and thus facilitate strategic and statistically sound disease surveillance sampling of wild feral swine populations specifically where foreign animal disease detection is critical.

**Time Sensitivity:** Feral swine populations in the US number over 5 million and are known to transmit or carry over 30 diseases and 37 parasites that can be transmitted to livestock, people, pets and wildlife. Current surveillance methods typically rely on samples extracted from removal operations. Though this methodology provides a large number of samples the data tends to be spatially and often temporally limited and is usually not tenable when specific surveillance sites must be targeted. As feral swine population numbers increase and they invade new landscapes the potential risk for disease transmission to domestic pig and other livestock operations increases. Thus developing additional sampling methods that can be rapidly deployed and provide usable diagnostic specimens would improve response time for surveillance and response operations.

#### 4. Development of Brucella vaccine for elk studies

**Collaborations:** USDA. ARS

**Description of objective or deliverable:** The deliverable for this series of studies is the development of a safe and efficacious oral brucellosis vaccine for use in elk in the field. We are experimenting with a killed elk strain *B. abortus* compounded with montmorillonite clay. An initial study in mice showed the killed *B. abortus* gave as good protection against intraperitoneal challenge as parenterally administered RB51. A second mouse study is underway to test protection against nasal-oral administered challenge.

The second component of this series of experiments is to utilize natural exposure to *B. abortus* as a challenge. This method of challenge, if successful, would be compliant with Select Agent rules and would allow vaccine studies to be done outside of BL 3 containment. Additionally, it would better simulate field conditions. To accomplish this, we are comingling pregnant seropositive elk with seronegative elk during winter and spring. If a shedding event occurs, we can observe how many naïve elk seroconvert and become infected. If a large percentage of comingled seronegative elk become infected, we can then conduct vaccine studies relying on natural exposure for challenge.

The third line of studies will examine protection of the mucosally administered killed *B. abortus* vaccine in elk using both natural challenge, if successful, and the classic intraconjunctival challenge in BL 3 containment.

**Benefit to VS program:** Brucellosis has been eradicated from cattle and ranched bison in the US except for the continuing spillback from elk. The infection in wild elk is expanding resulting in increased risk to more livestock herds and continuing enlargement of the Designated Surveillance Areas. Currently no vaccine is efficacious in elk and the Select Agent rules have virtually stopped vaccine experiments in elk. An ideal vaccine for use in wild elk would be administered remotely, on winter feedgrounds or on temporary feedlines established as a means of vaccinating elk. The vaccine would be killed, posing no risk to personnel, and dusted over the feed such that elk would encounter abundant mucosal exposure while feeding. If such a vaccine were successful in producing protection in elk, a vaccinated “firewall” of protected elk could stop the disease expansion in wild populations, followed by immunization of elk on feedgrounds aiding in the eradication of the infection in Greater Yellowstone Area wildlife and the nation.

**Time Sensitivity:** Currently, between 2 and 4 herds of cattle and ranched bison in the GYA are infected with brucellosis transmitted from elk, each year. There are few tools to mitigate this spread of the disease. These studies have been ongoing for 2 years and need to continue to completion.

#### 5. Development and evaluation of tuberculosis vaccine in feral swine

**Collaborations:** Dr. Richard Bowen, Colorado State University; Drs. Christian Gortazar and Joaquin Vicente, Instituto de Investigación en Recursos Cinegéticos (IREC), Universidad de Castilla-La Mancha, Spain, Drs. Ramon Juste and Iker Sevilla, Neiker-Tecnalia, Spain

**Description of objective or deliverable:** The deliverable for this series of studies is the evaluation and development of a safe and efficacious mucosal tuberculosis vaccine for feral swine in the field. Feral swine on Molokai Island, HI are believed to be a possible reservoir of *Mycobacterium bovis*, and feral swine along the border with Mexico and Texas are in danger of being exposed to *M. bovis*, both populations being potential sources of this pathogen to domestic livestock and other wildlife species. We

are experimenting with a killed *Mycobacterium bovis* administered orally to feral swine, shown by Spanish researchers to be effective in wild boar. A recent study comparing two strains of killed *M. bovis* given orally to Texas-origin feral swine with groups of feral swine either orally vaccinated with live BCG or non-vaccinated (control) did not show significant difference among the groups. In 2017 this study will be repeated, in Hawaiian origin feral swine, in order to determine if killed *M. bovis* works in that particular strain of pig. Further vaccine studies, initially in mice, and subsequently in feral swine will involve mucosal delivery of killed lyophilized *M. bovis* (see “Evaluation of killed preparations of *Brucella abortus* and *Mycobacterium bovis* in mice, elk, and feral swine).

**Benefit to VS Program:** Bovine tuberculosis is present in many wild swine populations throughout the world. In the United States, the disease is present in feral swine on Molokai Island, Hawaii, USA, which could serve as a potential source of *M. bovis* to cattle operations on the island. In addition, wild swine populations, present in areas where *M. bovis* prevalence in domestic cattle is high, such as in Mexico, could facilitate transmission of the disease into the United States, and have the potential to act as a reservoir population were a spillover from domestic livestock to occur. Therefore, tools such as effective mucosal vaccines that can be applied to wild swine in order to mitigate or eradicate *M. bovis* in those populations, would be of great benefit to the VS program.

**Time Sensitivity:** Feral swine on Molokai Island infected with *M. bovis* continue to threaten the domestic cattle herds. There is risk of spillover of *M. bovis* from domestic cattle to feral swine near the Texas border. We have been conducting vaccine studies in feral swine for two years, and must continue in order to develop an efficacious vaccination program in the country’s wild swine populations.

## **6. Development and evaluation of remote disease detection tools using volatile organic compounds**

**Collaborations:** Dr. Richard Bowen, Colorado State University; Dr. Radu Ionescu, Department of Electronics, Electrical, and Automatic Engineering, Universidad Rovira I Virgili; Drs. Christian Gortazar and Joaquin Vicente, IREC, Universidad de Castilla-La Mancha, Spain; Drs. Kurt VerCauteren and Christine Ellis, APHIS, Wildlife Services, National Wildlife Research Center; Dr. Jacek Koziel, Dept. of Agricultural and Biosystems Engineering, Iowa State University

**Description of objective or deliverable:** The deliverable for this series of studies is the evaluation and development of volatile organic compounds in breath and feces of wild swine, cattle, and white-tailed deer to detect bacterial and viral diseases in these species. We will collect breath and feces from captive and wild feral swine and wild boar naturally and experimentally-infected with *M. bovis* or pseudorabies virus, for VOC chemical and metabolomics analysis. We will also collect feces from cattle and white-tailed deer experimentally or naturally infected with virulent *M. bovis* for VOC analysis.

**Benefit to VS Program:** The detection of diseases in wildlife is generally done through techniques such as hunter-kill surveys, road-kill surveys, or actively capturing and/or killing animals for serologic testing and /or postmortem examination. There is need for less invasive, less expensive techniques to remotely detect disease in both wild and domestic animal populations. The detection of disease-specific volatile organic compounds (VOCs) from the breath and/or feces may provide a solution for remote surveillance of wildlife and offer rapid and efficient disease detection methods in livestock.

**Time Sensitivity:** There is great need for less invasive, less expensive techniques to remotely detect disease in both wild and domestic animal populations. We have been conducting VOC studies for six

years in various species and disease contexts. Research must continue in order to develop these methods into efficient and portable disease detection technologies.

## **7. Development of Safe and Effective Immobilization Protocols for Wild Swine**

**Collaborations:** Dr. Lisa Wolfe, Colorado Parks and Wildlife, Dr. Christine Ellis and Dr. Nathan Snow, APHIS Wildlife Services, National Wildlife Research Center, Dr. Clayton Hilton, Texas A and M. University-Kingsville, TX, Dr. William Lance, Wildlife Pharmaceuticals, Windsor, Colorado

**Description of objective or deliverable:** The deliverable for this series of studies is the evaluation and development of a safe, effective, and reversible protocols for the sedations and immobilization of feral swine in both a captive and field setting. A series of studies will fully evaluate various combinations of drugs for this purpose.

**Benefit to VS Program:** Scientists working with wild swine need improved methods to immobilize animals for purposes of applying collars, tags, and conducting disease research. These studies will provide comprehensive evaluation of various drug combinations in wild swine. Those combinations that appear most effective will be recommended for use by researchers and reported in peer-reviewed publications.

**Time Sensitivity:** This work will be carried out in the next two years and will need support for completion.

## **8. Evaluation of killed preparations of *Brucella abortus* and *Mycobacterium bovis* in mice, elk, and feral swine.**

**Collaborations:** Dr. Richard Bowen, Colorado State University

**Description of objective or deliverable:** The deliverable for this series of studies is the evaluation and of heat-inactivated, lyophilized *Brucella abortus* administered mucosally to mice in preventing infection or reducing disease severity caused by experimental exposure to virulent *B. abortus*. One study conducted in 2015 showed that lyophilized mucosally administered killed *B. abortus* offered protection in mice comparable to protection induced by RB51 administered IM in the face of IP *B. abortus* challenge. Further studies will continue investigating mucosally delivered lyophilized vaccines in mice. If successful, work will then be expanded to elk and swine, and it is hoped that effective brucellosis and tuberculosis vaccines will be developed for these species.

**Benefit to VS Program:** Bovine brucellosis has been successfully eradicated from livestock populations in the USA except for periodic outbreaks of the disease in cattle herds in the Greater Yellowstone Area (GYA) due to transmission from free-ranging elk. Elk and bison in the GYA are maintenance hosts and a continuing reservoir of *Brucella abortus* infection. While the current calfhood vaccine being used in cattle has been shown to be effective in bison, no vaccine to date has been shown to be effective in protecting elk from *Brucella abortus* infection or abortion. Swine brucellosis has also been successfully eradicated from livestock populations except for periodic outbreaks in transitional operations. The disease is endemic in many feral swine populations in the US and can be a source of outbreaks in outdoor swine operations as well as a source of infections in humans, such as pig hunters. Effective vaccines against *Brucella* infection in elk and swine would be very beneficial to US agriculture, wildlife, and

human health. Vaccines against bovine tuberculosis in feral swine are also greatly needed (see “Development and evaluation of tuberculosis vaccine in feral swine”).

**Time Sensitivity:** This work has been ongoing for approximately two years and will need support for at least the next 5 years.

#### **9. Use of Assisted Reproductive Techniques to Produce Brucellosis-free Bison with Yellowstone Genetics**

**Collaborations:** Colorado State University, Animal Reproductive Biology Laboratory

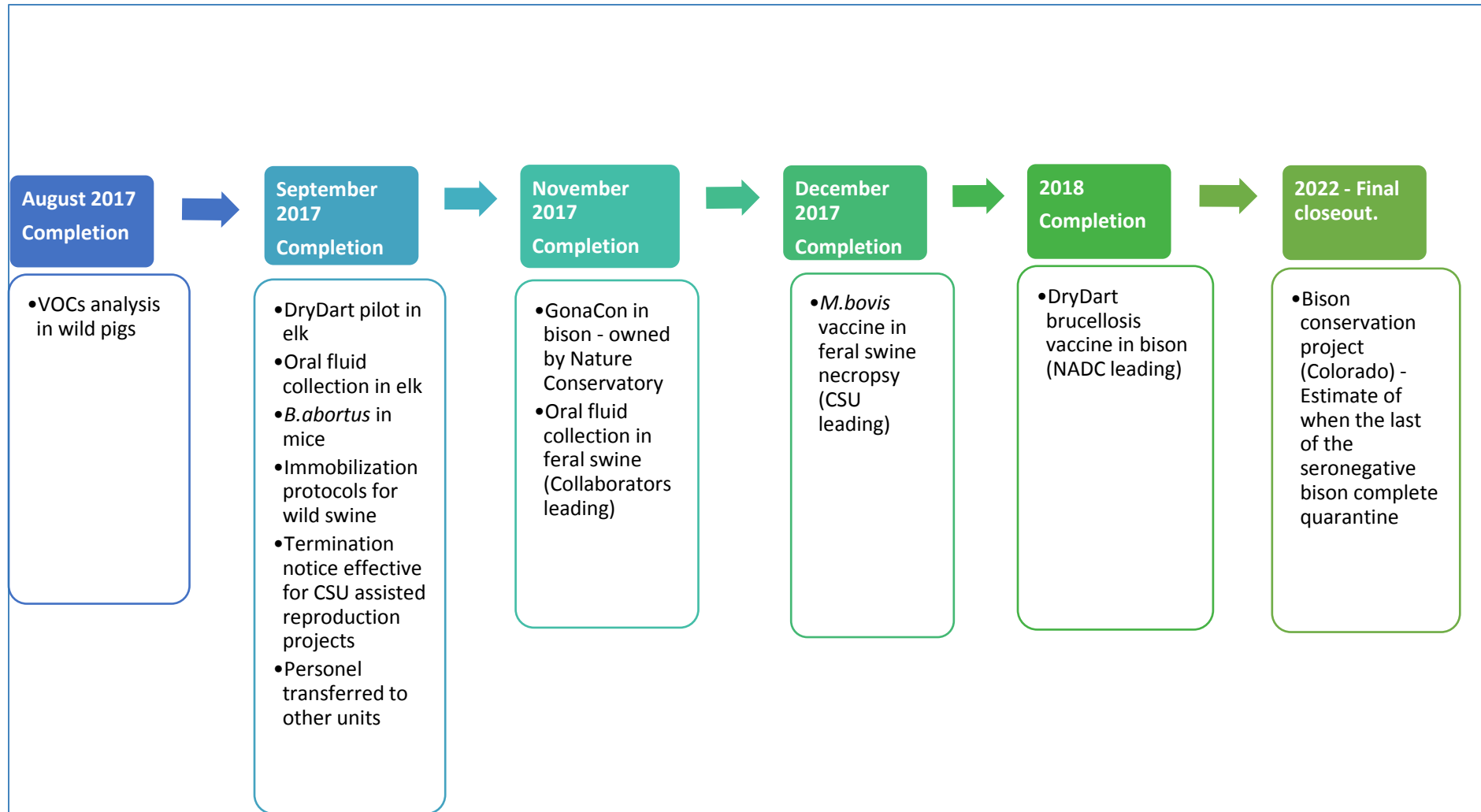
**Description of Objective or Deliverable:** WiLDIT is assisting CSU in this endeavor to salvage genetics from Yellowstone animals that go to slaughter or are kept in quarantine in order to establish herds of bison without cattle gene introgression that are free of brucellosis.

**Benefit to VS Program:** The establishment of pure, brucellosis-free herds remote from Yellowstone National Park should allow more disease management practices to occur in the Park bison.

**Time Sensitivity:** This work is in its 4<sup>th</sup> year and is having success. It likely will continue for another 5 years.

# WiLDIT Research Projects Completion Timeline\*

\*Disposition of all bison in MT is dependent on discussion with other entities.





Wildlife Livestock Disease Investigations Team Projects  
February 2017

**Current Projects:**

**-Development of GonaCon, an immunocontraceptive vaccine, as a method to stop *Brucella abortus* transmission in bison and elk.**

Brucellosis is a disease that is transmitted when the mother elk or bison has an abortion. This is a contraceptive that stops the mothers from getting pregnant. If they are not pregnant, they cannot abort. If they cannot abort, they cannot give the disease to other animals.

**-Development of DryDart, a vehicle to deliver lyophilized or powdered, pelleted medicaments to animals.**

A new darting technology that allows people to use a shotgun as a dart gun. The drugs in the dart are dry so they have a longer shelf life than liquid drugs.

**-Development of oral fluid collection studies for feral swine.**

Looking at a way to detect disease in feral pigs without having to catch them. We put out an interesting looking cloth ball, the pigs chew on it and get it wet with saliva. Then we can collect the ball, squeeze out the spit and test it to see what diseases the pigs have.

**-Development of *Brucella abortus* natural infection model in elk**

Brucellosis is spread when animals abort. We put pregnant brucellosis infected elk in a pen with elk that don't have brucellosis. If the sick elk have an abortion, they may give brucellosis to the other elk. If this works, we can do vaccine research and see if the vaccine works by putting brucellosis elk in with the vaccinated elk.

**-Evaluation of killed preparations of *Brucella abortus* and *Mycobacterium bovis* in mice, elk, and feral swine.**

We can take the bacteria that causes brucellosis or tuberculosis, kill it and put it in mist or a fine powder that can be breathed in by healthy animals. This may be a way of getting their immune system to protect them from the bacteria.

**-Development and evaluation of remote disease detection tools using volatile organic compounds**

We are looking at filters that will collect the breath of animals. We can then test the filters to see what kind of diseases those animals have.

**-Development of Safe and Effective Immobilization Protocols for Wild Swine.**

Using drugs to make pigs unconscious is difficult. We are looking at some new drug combinations that may be better. These include drugs that have an antidote so you can wake the pigs up when you are done.

Wildlife Livestock Disease Investigations Team Projects  
February 2017

**-Use of Assisted Reproductive Techniques to Produce Brucellosis-free Bison with Yellowstone Genetics.**

When Yellowstone animals that have brucellosis are sent to slaughter, they are lost forever. This involves making embryos from those animals and putting them in healthy surrogate bison. The result is that you have healthy Yellowstone bison calves born to bison mothers that don't have brucellosis.

## WiLDIT Strategic Plan

October 28, 2014

The mission of WiLDIT is to develop science-based solutions to disease problems at the wildlife/domestic animal interface.

To accomplish this mission, WiLDIT engages in 4 separate areas of activity or “strategies.”

1. Consultation – provide information and advice to USDA/APHIS and other State and Federal agencies and research partners on interface disease issues; serve as a liaison with agencies, universities and NGOs.
2. Developmental work – Coordinate and/or conduct developmental work to address VS-specific interface disease problem areas, i.e. vaccines and delivery systems for use in wildlife; remote screening and diagnostic techniques for use in wild populations; and strategies to detect, manage, and eradicate diseases in wild populations. Developmental work is usually accomplished through collaborations with other agencies and universities.
3. Training – Serve as a training resource for the agency concerning interface diseases.
4. Monitoring/Surveillance – Conduct surveillance of wild populations around disease outbreaks or on a continuing basis in endemic areas when requested.

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## Wildlife/Livestock Disease Investigations Team (WiLDIT)

### USDA/APHIS/VS, Western Region

#### Current Projects and Activities Update, August 2012

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4. BCG clearance in feral swine. Study designed to determine tissue clearance of orally administered BCG vaccine in feral swine. Study needed prior to eventual BCG field trial on Molokai. Collaborators: ARS, CSU.

Projects – Ongoing field/animal work

1. Oral feral swine vaccine studies using 2 candidate rough *Brucella suis* vaccines. Studies to determine protection of 2 candidate vaccines in feral swine. Collaborators: ARS, CSU.
2. Detection of volatile organic compounds (VOCs) in breath of animals infected with TB and brucellosis. Studies investigate effectiveness of VOC detection in the breath as a screening/diagnostic tool for TB and brucellosis. 2 TB studies showed promising results. Collaborators: APHIS-WS, ARS, CSU, Israel Institute of Technology.
3. GonaCon™ study in bison herd in southern Colorado. Study investigates safety and duration of infertility in bison vaccinated with GonaCon™ as potential tool to prevent *B. abortus* transmission. Collaborators: APHIS-WS, USGS- BRD.
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Other WiLDIT activities:

Extension/consultation, One Health participation, Consortium for the Advancement of Brucellosis Science (CABS) and USAHA Brucellosis Scientific Advisory Subcommittee members, instructors in CSU and APHIS courses, and host several externs each year.

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)

White-tailed deer: 13 owned & maintained by APHIS-WS for CWD studies at CSU

## Wildlife/Livestock Disease Investigations Team (WiLDIT)

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#### 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.
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**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*.

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5. Ecology and epidemiology of brucellosis in bear species in the GYA and the Arctic. Prospective and retrospective study of prevalence of brucellosis in polar bear and brown bears and investigation of origin of exposure/infection. Collaborators: USGS-BRD, NVSL
6. Venereal transmission of brucellosis in bison studies. Studies investigate transmission of *B. abortus* by venereal route in bison. First study showed seroconversion following intravaginal inoculation. Second study, a breeding trial, is ongoing. A contraceptive would not be effective in preventing shedding if venereal transmission is common in bison.
7. *Brucella abortus* infection/transmission model in elk. Project to start in winter/early spring 2013. A natural infection/transmission model will be tested in *B. abortus*-infected wild elk obtained from the GYA. If successful, this model will be used for subsequent vaccine studies in this species.
8. Molokai feral swine colony. Establish breeding colony of feral swine originating from Molokai-winter/early spring 2013. These swine will be used for the purposes of tuberculosis vaccine studies.

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Commented [pn2]: Take or leave as well

Other WiLDIT activities:

Extension/consultation, One Health participation, Consortium for the Advancement of Brucellosis Science (CABS) and USAHA Brucellosis Scientific Advisory Subcommittee members, instructors in CSU and APHIS courses, and host several externs and visiting scientists each year.

Census of Animals in Fort Collins Facility

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

Commented [pn3]: How many are there currently?

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

White-tailed deer: 13 owned & maintained by APHIS-WS for CWD studies at CSU



## Wildlife Livestock Disease Investigations Team Brucellosis Research Update

The Wildlife Livestock Disease Investigations Team (WiLDIT) under VS, STAS, NVSL has been devoted to developing science-based solutions to disease problems at the wildlife/domestic animal interface since 1997. Tools for management of brucellosis in wildlife have been an important focus of the group since its inception. WiLDIT approaches the many challenges associated with managing wildlife brucellosis through filling vitally important knowledge gaps and exploring different management strategies that may be applied with complementary or synergistic effects. Currently WiLDIT's brucellosis projects involve the following: Immunocontraception, Vaccination, Detection, and Conservation.

### Immunocontraception

In female bison, brucellosis is transmitted if pregnancy occurs. In over 300 bison cow captures, *Brucella abortus* was isolated from the vagina, milk, blood, feces, and products of parturition. WiLDIT has been researching a GnRH-based immunocontraceptive vaccine, GonaCon™, that produces long term infertility in female bison. Two studies were initiated in 2011 evaluating GonaCon™ for efficacy in bison. One study is being conducted in Great Sand Dunes National Park looking at duration of infertility in bison. Ten bison cows were vaccinated and ten cows were not vaccinated, and all 20 animals were monitored every year for calf production. This study will be completed in the fall of 2017. The results through 2016 are as follows:

Number pregnant/number in group (percent pregnant)

	Nov 2011	Nov 2012	Nov 2013	Nov 2014	Nov 2015
Treatment	4/10 (40)	3/9 (33)	1/10 (10)	3/9 (33)	3/10 (30)
Control	4/10 (40)	9/9 (100)	6/9 (67)	9/9 (100)	6/9 (67)

At the Bison Quarantine Facility (BQF) in Corwin Springs, MT, in close collaboration with SPRS scientists, the project "Management of *B. abortus* in bison through immunocontraception" was initiated in 2011. Brucellosis serology-positive bison cows were collected from Yellowstone National Park and housed at the BQF. The animals were placed in two separate pastures, one group was vaccinated with GonaCon™ and the other group was not vaccinated. Sentinel brucellosis serology-negative bison were placed in both pastures as well to monitor for transmission events. All cows in both pastures were exposed to negative bulls during breeding season and then removed afterwards. A second iteration of the study was started in 2014. The following tables show the results through 2016 regarding the efficacy of GonaCon™ in preventing pregnancy in bison.

## Number pregnant/number in group (percent pregnant)

### First Cohort

Group	2013	2014	2015	2016
Treatments	3/15 (20)	2/15 (13)	5/14 (36)	3/14 (21)
Controls	11/14 (79)	10/13 (77)	10/12 (83)	10/12 (83)

### Second Cohort

Group	2015	2016
Treatments	1/20 (5)	5/19 (26)
Controls	n/a	13/21 (62)

In non-vaccinated groups of both iterations, infectious abortions occurred and transmission was documented. In contracepted animals, no transmission events have been documented, thus supporting the idea that preventing pregnancy will stop transmission of brucellosis. It is interesting to note that, contrary to traditional beliefs about the disease, some bison cows experienced multiple abortion or shedding events. It is widely believed that it is only the first calving after *B. abortus* infection leads to abortion, still birth, or shedding. This is not what we observed in this study and is extremely important information when making management decisions regarding a brucellosis positive bison population.

### Vaccination

Another management strategy for controlling brucellosis in wildlife is remote vaccination via darting or oral/mucosal uptake. WiLDIT is working on several technologies to this purpose.

#### DryDart

The latest data produced by ARS researchers have shown that the brucellosis vaccine RB51 is highly effective in protecting bison from the disease when given in two doses 1 year apart. WiLDIT personnel have developed a biodegradable darting technology that delivers lyophilized, powdered, or pelleted vaccines capable of being fired from a dart gun or shotgun. This type of dart can be used in the field to effectively vaccinate bison with RB51.



#### Mucosal vaccination with powdered, killed vaccine

WiLDIT is working with collaborators to develop an effective delivery method for oral and other mucosal vaccines in powdered form. Current research is exploring lyophilized, killed *B. abortus* adhered to a fine particulate clay that is administered to mice via oral, respiratory, and ocular exposure. Data have shown so far that mice develop protection against *B. abortus* infection with this vaccine construct similar to RB51. Follow up studies are underway and will also be tested in elk in the near future. This vaccination strategy, if effective in a controlled setting, could be administered to hay on Wyoming feedgrounds in order to vaccinate wild elk populations with brucellosis.

#### Detection

Disease detection methods for free-ranging wildlife currently require that the animals be handled or killed. WiLDIT is working on developing detection methods that can be deployed remotely without having to handle any animals. Two technologies currently being explored involve collection of volatile organic compounds (VOC's) from breath and other biological samples, and collection of oral fluids.

#### VOC's

VOC's can be collected on adsorptive materials that have been exposed to breath or headspace of biological samples, such as feces. The collected VOC's are then analyzed via gas chromatography/mass spectrometry, or via nanosensor technology. Two studies were conducted in collaboration with SPRS scientists and researchers at the Technion in Israel, with breath collected from brucellosis-positive and negative bison. These preliminary studies have indicated that good discrimination between positive and negative animals can be achieved. In addition, environmental factors did not appear to affect the outcome of the statistical models applied to the data, and results were consistent over time.

#### Oral Fluids

Oral fluids can be used to detect evidence of disease exposure through analyzing for presence of nucleic acids and antibodies. WiLDIT researchers are currently exploring ways to collect oral fluids from wild swine and elk in order to analyze the collected oral fluids for evidence of brucellosis. Structures that encourage chewing by either swine or elk, and are capable of collecting and preserving the integrity of oral fluids are being tested in controlled and field settings

#### Conservation

As part of WiLDIT's agreements and IACUC protocols, Yellowstone bison that are test negative for brucellosis are used for conservation purposes after they are subjected to the brucellosis quarantine process as defined in the UM&R. To date five Yellowstone bulls have been transferred to the USFS at the Midewin National Tallgrass Prairie. Also, through a collaboration with Colorado State University, The City of Fort Collins, and Larimer County, the Laramie Foothills Bison Conservation Herd has been established using bison that have passed the requirements for quarantine including three cows and four calves from the Gonacon study. This herd is currently comprised of 23 animals.

## WiLDIT Work Plan FY2015

### Feral Swine Work:

Continue pig TB and *Brucella* vaccine work. Specifically, obtain Hawaii pigs and start colony. Conduct Texas pig trial with killed *M. bovis*. Plan for next *Brucella* trial.

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Collect VOCs from pigs in experimental infections, Spanish wild boar, and any other field opportunities that arise.

Conduct oral fluid test to see which bait is most attractive. Collect oral fluids from pigs as opportunities arise.

Begin pathogenesis and vaccine work on FMD, CSF, and/or ASF.

### Elk Work

Repeat *Brucella* natural exposure model experiment.

Continue work on spray-dried vaccine for *Brucella*

Explore surveillance work on or around Hardware ranch

### Bison Work

Continue GonaCon studies in MT and at Great Sand Dunes

Compile data for John Eisemann to pursue getting GonaCon registered for bison ~~on-label~~

Continue Drydart development – small bison and elk study

Pursue GTNP and/or YNP brucellosis vaccination field trial

Continue to assist CSU on assisted reproductive techniques work

### Other

Mouse studies evaluating spray-dried *Brucella* vaccines

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Assist Torsten Eckstein with lipid ELISA development

Polar bear work



Ecology of Fish brucellosis

Various other collaborations beneficial to VS (as they arise)

## **WiLDIT Work Plan FY2015**

### **Feral Swine Work:**

Continue pig TB and *Brucella* vaccine work. Specifically, obtain Hawaii pigs and start colony. Conduct Texas pig trial with killed M.bovis. Plan for next *Brucella* trial.

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### **Elk Work**

Repeat *Brucella* natural exposure model experiment.

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### **Bison Work**

Continue GonaCon studies in MT and at Sanddunes

Compile data for John Eisemann to pursue getting bison on label

Continue Drydart development – small bison and elk study

Pursue GTNP and/or YNP brucellosis vaccination field trial

Continue to assist CSU on assisted reproductive techniques work

### **Other**

Assist Torsten with lipid ELISA development

Polar bear work

Fish brucellosis

## Joint WiLDIT and GYA

### Current Projects and Activities Update, August 2012

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

1. Investigation of seminal shedding of *B. abortus* by Yellowstone bulls in the spring. The study demonstrated shedding of *B. abortus* in the semen of Yellowstone bulls in the spring and evaluated semen quality. Collaborators: Montana Department of Fish, Wildlife, and Parks
2. Bison Quarantine Feasibility Study. Study demonstrated successful graduation of seronegative, culture negative bison by the diligent use of the bison quarantine protocol published in the UM&R. Collaborators: Montana Department of Fish, Wildlife and Parks.

Commented [PN1]: I suggest this because they helped out in the field?????

Projects – Ongoing field/animal work

1. Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means to decrease shedding of *B. abortus* in bison. Project will evaluate shedding of *B. abortus* in contracepted and control bison over 6 years. It will also determine outcomes of calves born to positive cows and evaluate shedding of contracepted cows after fertility returns. Collaborators: APHIS-WS, ARS, NPS.