

**National Wildlife Research Center**  
**Standard Operating Procedure**

<b>Title:</b>  Study Protocols	<b>Number:</b> AD 003.04 <b>Effective date:</b> 6 Dec 2010
	<b>Replaces:</b> AD 003.03
	<b>Management approval:</b> 12/6/10 [Signature] Date
<b>Prepared by:</b> Catherine Bens, [Signature] Laura Greiner [Signature]	<b>Date:</b> 12/6/10 12/3/10 <b>Processed by QAU:</b> [Signature] <b>Date:</b> 12/6/10

**1.0 Purpose**

To provide guidelines for the writing, review, approval and revision/change of study protocols.

- 1.1 Study is any structured scientific activity that requires committed NWRC resources such as personnel, facilities, equipment, funds.
- 1.2 Study activities requiring an approved protocol include, but are not limited to exploratory, preliminary and definitive research, formalized training, student theses and dissertations, statistical and economic analysis, operational program support activities, contracted research, or other activities requiring the use of animals, bio/hazardous materials, or specimen archiving.
- 1.3 **Studies conducted by, or under the auspices of the NWRC may not be initiated until all signatures, including that of the Director's Office, have been obtained.** Approval or exemption from IACUC or other institutional oversight still requires a fully authorized protocol, including signature from the NWRC Director's Office.

**2.0 Authority**

Code of Federal Regulations (CFR)

40 CFR Part 160: Good Laboratory Practice Standards (FIFRA)

40 CFR Part 792: Good Laboratory Practice Standards (TSCA)

21 CFR Part 58: Good Laboratory Practice Standards for Nonclinical Laboratory Studies (FFDCA)

9 CFR Parts 1-4: Animal Welfare Regulations

NIH Guidelines for Research Involving Recombinant DNA Molecules

### 3.0 Attachments

Attachment 1: Protocol and appendices templates  
Attachment 2: Protocol amendment template  
Attachment 3: Note to file template  
Attachment 4: NWRC Protocol Decision Tree

### 4.0 Procedure

#### 4.1 Protocols

##### Step 1: Protocol number assignment

Study protocol numbers will be assigned by the Quality Assurance Unit (QAU). Please provide the following information by email or phone call:

- Name of study director
- Protocol title
- Regulatory compliance status (non regulated, EPA GLP, FDA GLP, or other)
- NWRC Project title

##### Step 2: Protocol preparation and review

Prepare a study protocol in the style, content, and format of Attachment 1. Normally this is done by or under the direction of the Study Director. Delete directions provided within the protocol template as appropriate.

Distribute an electronic copy in **draft** form to the appropriate reviewers. **No signatures are required during this step.** At a minimum, the protocol must be posted to the NWRC intranet site for 2 weeks (in the *Draft Protocols* folder). To add a new document to the intranet, go to this site:

<http://animalhealth/nwrc/ga/protocolws/default.aspx>

Generally, the Study Director provides copies simultaneously to the appropriate reviewers; however this is not required. The Study Director may have as many reviewers as he/she deems necessary, but NWRC policy and the Good Laboratory Practice regulations require review by at least the following:

- **Project Leader and Assistant Director:** To ensure that the study meets requirements of the Project and to assure that adequate funding, sufficient personnel and necessary resources are available for the timely and proper conduct of the study. *Review is required by NWRC policy.*
- **Sponsor(s):** APHIS is the sponsor for the majority of the studies done by NWRC and sponsor approval is obtained with the Director's Office signature. If the sponsor is other than APHIS, have the sponsor review and approve the protocol. Sponsor approval, including the date of approval, should be obtained and documented via a signature in the



protocol, letter, facsimile, e-mail or otherwise. If a non-APHIS sponsor elects not to review the protocol, the date of approval would be the date of the contract or agreement between the sponsor and NWRC. *Sponsor approval of the protocol and any changes is required by regulation 40 CFR, Part 160, §160.120.*

- **Quality Assurance Unit (QAU):** To ensure proper format and compliance with regulations and policies, and to facilitate tracking of the protocol, study progress and final reports as per this SOP and regulation 40 CFR, Part 160, §160.35.
- **IACUC, IBC, and Individual Specialist Reviews:** Review by the Institutional Animal Care and Use Committee, Institutional Biosafety Committee, or individual specialists (e.g., analytical chemist, formulation chemist, consulting veterinarian, statistician, NEPA specialists) may be necessary as specified in the protocol format.

### **Step 3: Comments and Revision**

Comments and suggested changes from reviewers should be addressed with appropriate changes to the protocol or other documentation. The Study Director should return revisions or responses to the reviewer to satisfy questions, comments, suggestions, or concerns about the draft protocol.

### **Step 4: Obtaining Approvals**

Once all changes have been made, the Study Director should sign and date the finalized protocol, obtain appropriate signatures and forward the **original** hard copy and an electronic Word copy to the QAU for final processing.

The QAU will forward the protocol to the Director's Office for approval. The Director's Office will return the approved protocol to the QAU for distribution. **Approval by the Director's Office authorizes start of the study and release of the protocol for distribution to participants and use in the laboratory or field.**

The QAU will retain the **original** of the approved protocol, post a scanned copy to the NWRC intranet and provide a copy to the Study Director and the NWRC Attending Veterinarian.

### **Step 5: Scheduling Inspections**

It is the responsibility of the Study Director to keep the QAU informed of the study schedule in a timely manner in order that QA may appropriately schedule study inspections as required by regulation. The onus is on the Study Director to keep QAU informed of study progress and status.

4.2 **Protocol Amendment/Change/Revision**

Studies will be conducted in accordance with the protocol. However, circumstances may dictate that changes are needed.

Use the *Protocol Amendment/Change/Revision form* (Attachment 2) to document changes in or revisions of an approved protocol. Document permanent, planned changes in study design, correct obvious errors in the protocol, change schedules, or resolve relatively minor, unplanned changes in study conduct.

Note: Changes involving the use of animals must be approved by IACUC and Management before that change is implemented.

**Amendments/Changes/Revisions to a protocol must be signed and dated by the Study Director, Project Leader, Assistant Director, Sponsor and committees as needed, and the original forwarded to the QAU for processing, along with an electronic Word copy.** The QAU will retain the original, post a scanned copy to the NWRC intranet and provide a copy to the Study Director and the NWRC Attending Veterinarian.

4.3 **Standard Operating Procedures (SOP) and SOP Deviations**

Procedures described in protocols override SOP procedures. Minor deviations from associated SOPs sometimes occur during the conduct of a study (e.g., weighing of an animal was missed one day; the time of a daily observation was not recorded). Such changes shall be authorized by the study director and documented as a Note to File (Attachment 3) or similar documentation.



United States Department of Agriculture  
Animal and Plant Health Inspection Service/Wildlife Services  
National Wildlife Research Center  
**PROTOCOL COVER PAGE**

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

### PROTOCOL CLASSIFICATION

<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".

---

**PART ONE: SIGNATURE PAGE**

---

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

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

Position (check one):

☐ Biologist/Chemist/Technician  
Supervisor signature required:\_\_\_\_\_ Date \_\_\_\_\_ ☐ Res. Scientist ☐ Proj. Leader☐ Research Scientist☐ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: \_\_\_\_\_☐ Student: NWRC Representative/Contact: \_\_\_\_\_

Concur:

NWRC Research Project Leader \_\_\_\_\_ Date \_\_\_\_\_

Review and Processing:

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

Concur:

NWRC Assistant Director \_\_\_\_\_ Date \_\_\_\_\_

Approved:

NWRC Director \_\_\_\_\_ Date \_\_\_\_\_

---

Note: Additional approvals are located in the attached appendices.

---



## PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item						
<b>Animal Use</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.</p> <p><input type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach <b>NWRC Animal Use Appendix</b></p> <p><input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach <b>IACUC protocol &amp; approval</b></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>Microbiological/Biohazardous Materials</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach <b>Microbiological/Biohazardous Materials Use Appendix</b>.</p>						
<b>Permits</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates.</p> <table style="width: 100%; border: none;"> <tr> <td style="border-bottom: 1px solid black; width: 50%;"></td> <td style="border-bottom: 1px solid black; width: 20%;"></td> <td style="border-bottom: 1px solid black; width: 30%;"></td> </tr> <tr> <td style="text-align: center;">Permit(s) description</td> <td style="text-align: center;">Number</td> <td style="text-align: center;">Date</td> </tr> </table>				Permit(s) description	Number	Date
Permit(s) description	Number	Date						
<b>National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the <b>NEPA &amp; ESA Appendix</b>.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the <b>NEPA &amp; ESA Appendix</b>. Contact QA/NEPA staff for ESA or eagle incidental take requirements.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.</p>						
<b>Regulatory Standard and Test Guidelines</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study be conducted under any regulatory standard? If yes please check:</p> <p><input type="checkbox"/> <i>CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)</i></p> <p><input type="checkbox"/> Other: _____</p>						
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline: _____</p>						
<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 <b>Test, Control and Reference Material/Devices Formulation and Use Appendix</b>. Please indicate if otherwise described in the protocol.</p>						
<b>Historical Resources</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.</p>						
<b>Material Transfer Agreement</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate <b>Material Transfer Agreement</b>.</p>						
<b>Analytical Chemistry</b>								
<input type="checkbox"/>	<input type="checkbox"/>	<p>Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?</p> <p>If yes, attach <b>Analytical Chemistry Appendix</b>.</p>						



**PART THREE: DESCRIPTION OF ACTIVITIES**

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

Collaboration:	Name	Address or Organization	Role in Project

Start Date:

End Date:

Archive Date:

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

Materials to be archived to close this activity:

Description of Project and NWRC Activities and Participation:

Comments:

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

**PART FOUR: FULL NWRC STUDY PROTOCOL****1. Key Personnel**

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

**2. Testing Facilities**

Name	Address	Role in Study

**3. Sponsor**

Name	Address	Contract No.

**4. Schedule**

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

**5. Background and Justification**

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

**6. Related Protocols**

List by Protocol Number

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

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

## 8. Objective/Hypotheses

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

## 9. Methods/Procedures

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

## 10. Experimental Design and Statistical Analyses

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

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

SOP/Method No.	Title

## 12. List of Records to be Maintained

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



### 13. Cost Estimate for Each Fiscal Year

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

### 14. Human Health and Safety

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

### 15. Staff Qualifications

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

### 16. Archiving

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

### 17. Protocol Amendments

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

### 18. References

List in alphabetical order by author.

---

## 19. Appendices

Indicate none or check attached appendices:

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

## Animal Use Appendix

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

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

### A. Animal Description

#### 1) Animals:

Species, subspecies (if applicable):

Breed, strain and substrain (if applicable):

Total Number and Sex:

Body weight range:

Age:

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

1) Rationale for involving animals:

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

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

### C. Source

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

### D. Method of identification of animals

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

### E. Trapping/Collecting

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

### F. Transport

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



**G. Handling/restraint**

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

**H. Quarantine**

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

**I. Housing/maintenance**

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

**J. Dietary contaminant exposure**

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

**K. Disposition of animals**

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

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

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

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

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

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

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

☐ No

☐ Yes If yes, continue with the following items.

**a) Alternative procedures:**

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

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

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

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

c) Surgery:

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

#### **M. Euthanasia**

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

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

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

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

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

---

### Analytical Chemistry Appendix

---

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

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

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



---

**Column E Explanation**

---

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

Agency:

CFR:

# Material Transfer Agreement

---

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

## **PARTIES:**

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

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

## **PURPOSE:**

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

The Material is released to Recipient under the following conditions:

1. The Material and associated know-how shall only be used for [give the specific purpose(s) that the material may be used for].
2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.
3. The Material shall remain the property of APHIS and shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.
4. Recipient shall keep APHIS informed of the results obtained through your use of the Material and shall provide APHIS with any manuscript that describes the work with the Material prior to submission for publication and acknowledge APHIS' contribution to the work reported.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health and Inspection Service, the Center for Disease Control, and /or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.

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

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

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

QA#: Permit Information  
(Type and Number):

Typed name/Title

Signature (NWRC APHIS Scientist)

Date

Typed Name/Title

Signature (NWRC APHIS Assistant  
Director)

Date

**ACCEPTED FOR RECIPIENT:**

Typed Name/Title

Signature

Date

**APPROVED:**

Typed Name/Title

Signature (Technology Transfer  
Coordinator)

Date

Original: NWRC Agreements Specialist

cc: Technology Transfer Program Manager, Quality Assurance Unit



# Material Transfer Agreement

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

## PARTIES:

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

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

## PURPOSE:

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

*[Table may be adjusted as needed]*

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

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

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

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

## ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

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

Typed name/Title

Signature (NWRC APHIS Scientist)

Date \_\_\_\_\_

Typed Name/Title

Signature (NWRC APHIS Project Leader)

Date \_\_\_\_\_

ACCEPTED FOR RECIPIENT:

Typed Name/Title

Signature  
(Technology Transfer Coordinator)

Date \_\_\_\_\_

Original: Quality Assurance Unit



---

## Microbiological/Biohazardous Materials Use Appendix

---

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

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at [www.cdc.gov/od/ohs/biosfty/biosfty.htm](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: \_\_\_\_\_

Study Director: \_\_\_\_\_ Amendment No.: \_\_\_\_\_ Page \_\_\_\_ of \_\_\_\_

Study title: \_\_\_\_\_  
\_\_\_\_\_**Changes in schedule:**

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

**Protocol section/subsection/appendix to be changed:****Description of revisions:** *(Please provide the level of detail normally required in the protocol)***Justification/reason(s) for changes and impact on study:** *(If dates are changed, please provide a description of current status of study and remaining study plan/schedule.)*

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

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

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

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

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

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



United States  
Department of  
Agriculture



Animal and  
Plant Health  
Inspection  
Service

Wildlife Services

National Wildlife  
Research Center

**Date:**

**Subject:** Note to file for QA-\_\_\_\_\_

**To:**

**Copies to:**

---

**Signature**

---

**Date**



AD003.04

*Safeguarding American Agriculture*

APHIS is an agency of USDA's Marketing and Regulatory Programs

An Equal Opportunity Provider and Employer

001796

## NWRC Protocol Decision Tree

Are NWRC staff involved in study design, data collection, experiments, or animal studies?

**NO**

Activities do not involve regulated research activities\* and are generally limited to NWRC personnel time

### Classification 1

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 3 (Description of Activities)

### EXAMPLES:

- Writing or collaborating on review papers and synthesis reports
- Student committee participation
- Analyzing or writing up data collected under operational or other contexts without prior input from NWRC scientists

**YES**

Activities involve regulated research activities\*

NWRC activities involve regulated research activities\*.

### Classification 2

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 3 (Description of Activities)
- ✓ NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval, as applicable.
- ✓ NWRC Material Transfer Agreement (Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable)

### EXAMPLES:

- Training programs requiring the use of animals
- Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required)
- Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)

### Classification 3

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 4 (full NWRC Study Protocol)
- ✓ Collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.

### EXAMPLES:

- Collaborating on study design, data analysis, or economic analysis.
- Minor participation on a regulated study at the collaborating host institution
- A study that does not include animal use, etc.

### Classification 4

Submit the following:

- ✓ Cover Page
- ✓ Part 1 (Signature Page)
- ✓ Part 2 (Regulatory Considerations)
- ✓ Part 4 (full NWRC Study Protocol)
- ✓ Required documents under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.

### EXAMPLES:

- A typical NWRC led study
- Major NWRC staff participation in regulated activity
- Study takes place on NWRC facilities

\* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use"

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

6. Procedures for Handling Infected or Restricted Herds

D. Approved Bison Quarantine Facilities

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

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

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

All bison entering an approved bison quarantine facility are considered to be brucellosis-exposed animals and must be permanently identified with official metal eartags and RFID tags and placed under quarantine restrictions. Each bison must have at least two permanent forms of identification prior to entering an Individual Test Group (ITG). Prior to entering the facility, all animals must test negative on official brucellosis serological tests conducted at the National Veterinary Services Laboratories (NVSL) or at an approved Cooperative State-Federal Brucellosis Laboratory (CSFBL). All serological and/or milk tests conducted in the ABQF are considered preliminary and must be confirmed at NVSL or at an approved CSFBL. (b)(3)

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



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

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

(b)(3)

Any bison removed from the ABQF before completing the requirements to qualify for quarantine release must move under permit either to an approved research facility or to an approved slaughter facility for slaughter only. (b)(3)

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

### 1) Males

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

### 2) Breeding bulls

- (a) Bulls must (b)(3)

be from an ITG that has qualified for a quarantine release.

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

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

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

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

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

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

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

**5) Calves**

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

6) (b)(3)

(b)(3)

## 7) Neutered animals

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

## 8) Post-quarantine requirements

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

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

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



## IMPLEMENTATION PLAN TO DISBAND WILDIT

### SUMMARY

This plan addresses the request from the Office of the Deputy Administrator for a plan to immediately ramp down the work of WildIT, close the research facilities at the NWRC in Fort Collins and the pens at Corwin Springs, Montana 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 Understandings (MOU) in place with other entities regarding the project and disposition of the research animals is included. Personnel will be moved out of the WildIT group by the end of FY17. The disposition of all animals will be determined by June 30, 2017. In a few cases, discussions with project collaborators are needed after approval of the implementation plan to determine if they would want to acquire the research animals. None of the animals that would be transferred would be positive for brucellosis. The seropositive and seronegative bison at the MT facilities would be disposed of per agreements with the National Park Service. Colorado State University would receive the sixty day termination notice provided for in the MOU regarding xxxx.

It has been determined that a 1010 package and Congressional notification are not required for this organizational change, however, a Civil Rights Impact Analysis is needed.

### PERSONNEL PLACEMENT

The four permanent employees currently assigned to the WildIT group will retain their grades and be reassigned pending approval from the Civil Rights Impact Analysis which is currently underway. The term employee is in the second year of a possible four-year position which expires March 6, 2018. There is one Saul T. Wilson student (b) (6). There are xxxx8 part-time students hired through CSU which will conclude employment by, xxxJuly 31, 2017.

- Dr. Jack Rhyan will be assigned to NVSL to lead 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. His duty station would remain in CO.
- Dr. Pauline Nol, Veterinary Medical Officer, and Matt McCollum, Wildlife Biologist, will be reassigned to CEAH to provide wildlife expertise to CEAH projects. They are meeting with the CEAH Director to determine the best organizational unit at CEAH to be reassigned to.
- Samantha Bruce, the Saul T. Wilson student, will transfer to CEAH. Since [REDACTED]
- Karl Held, Animal Health Technician, will be assigned to the SPRS District 6 office in Lakewood, CO, with his duty station as his home.

Commented [LEA-A1]: Please fill in the two XXX areas. Do we actually hire them or CSU does and then do we reimburse somehow?

- 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 (b) (6)

She is meeting with the CEAH Director to determine where her skills would best be used. WS may have at least partial salary funding to allow completion of a feral swine collaborative project.

## RESEARCH PROJECTS WRAP-UP

### Colorado Projects

**Project:** Evaluation of duration of infertility produced by GonaCon, an immuno-contraceptive vaccine, in bison.

**Collaborators:** The Nature Conservancy; WS/NWRC

**Location:** (b) (6) (animals owned by The Nature Conservancy) in southern Colorado.

**Status:** Started in 2011 with brucellosis negative bison and to be completed in November 2017. APHIS does not have ownership of these animals. We will visit with the owners of the animals (Nature Conservancy) and NWRC about ending this project early. If not, Jack Rhyan will discuss transfer of those duties with NWRC.

**Agreements:** Evaluation of GonaCon (See document Protocol QA1923). This agreement requires tissues be taken for histopathology. Jack Rhyan will do the histopathology examination. Writing this project up will be done jointly with NWRC and Jack Rhyan. This study is being conducted under NWRC approved Protocol QA1923.

**Project:** "Brucellosis infection and transmission dynamics in elk."

**Collaborators:** WYG&F, ARS, CSU

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Project was halted in February of 2017 prior to mixing seronegative and seropositive elk that were collected in 2017, and all seropositive elk were removed by April, 2017.

Collaborators were notified in February and March of 2017. This project did not have any agreements with collaborators. A CSU approved IACUC protocol was in place (#14-4956A). Collection permits were in place with Wyoming Game and Fish (#33-1040-2017; #33-1041-2017) and Department and Colorado Parks and Wildlife (#17TR2152)

**Project:** "Development of DryDart technology to deliver brucellosis vaccine to bison"

**Collaborators:** ARS/NADC

**Location:** ARS/NADC WiLDIT Wildlife Research Facility, Fort Collins, CO

**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.

**Commented [LEA-A2]:** Any type of graphic we can do on the timeline for projects in addition to the text? Maybe a CEAH technical writer can help us Mon.

**Commented [LEA-A3]:** Is this the Nature Conservancy Ranch?

**Commented [LEA-A4]:** How did we know they were seronegative? Did they go through quarantine procedures somewhere?

**Commented [NP-A5R4]:** These animal are from a brucellosis negative herd. For this study their status is irrelevant so no need for this statement

**Commented [LEA-A6]:** Is there any impact on study results by ending before end of Sept vs. November?

**Commented [NP-A7R6]:** These animals are only rounded up in November of each year. If they want to keep the animals then there is no impact. If we have to arrange for them to be euthanized then that will be problematic to do so before November

**Commented [LEA-A8]:** Where did the bison for this study come from?

**Commented [NP-A9R8]:** They are animals on the premises and are owned by the Nature Conservancy

**Commented [LEA-A10]:** Do you have this protocol in case asked for it?

**Commented [NP-A11R10]:** Now in document

**Commented [LEA-A12]:** Is this accurate?

**Commented [NP-A13R12]:** All elk collected from WY in 2017 were in same pen, and it turned out they were a mixture of positive and negatives. And Non pregnant seropositive elk from 2016 were mixed with seronegatives. I suggest leaving this statement out because it is misleading and not useful in this document.

**Commented [LEA-A14]:** Do we need a statement about how determined status so might have mixed seropos with seroneg?

**Commented [NP-A15R14]:** Is this necessary to even discuss here? I suggest leave it out because it is confusing and this issue has been resolved.

**Formatted:** Font: (Default) Times New Roman, 12 pt

**Commented [LEA-A16]:** Is the study at NADC?

**Commented [NP-A17R16]:** Yes as stated in document

An additional pilot study to evaluate the use of DryDarts to deliver immobilizing agents to animals ~~will be~~ is being conducted at the WiLDIT facility in Fort Collins in summer 2017 with completion before the end of ~~but will be complete by the end of~~ September 2017.

**Project:** “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:** There is an MOU that requires a 60 day notice for termination. This project used bison owned by APHIS that will now be no longer available for use by CSU. These bison are also under the four agreements applicable to YNP bison.

**Commented [LEA-A18]:** What type of animals? Anything seropositive? Is it in Fort Collins?

**Commented [NP-A19R18]:** I'll have to touch base with Jack on this

**Project:** “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 TB-free herd. At this time, these animals will no longer be the primary responsibility of WiLDIT. A master's student is dependent on having these animals available for sampling in the BSL-3 for her oral fluids project (Two Agreements: Alfano, Marialexia-Learning Plan; Alfano, Marialexia-Placement Agreement”). Animals will be necropsied November/December 2017. Disposition of pigs is 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).

**Commented [LEA-A20]:** Can we put something on disease status of these animals? Are they brucellosis and TB negative?

**Commented [NP-A21R20]:** These animals are brucellosis and TB negative based on disease free parentage. The herd is disease free

**Project:** “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:** – Current cooperative agreement with Rovira i Virgili University will be completed and closed out by ~~XXXX~~ 14 August, 2017 and manuscript(s) prepared and submitted for publication based on data collected from previous projects. Intended sample collections from current projects will be taken over by NWRC or will not take place.

**Commented [LEA-A22]:** Can we put date?

**Project:** “Development of oral fluid collection studies for feral swine”

**Collaborators:** CSU, VS-SPRS; University of Florida; University of Georgia.

**Locations:** Savannah River Field Station, GA 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 University of GA and UFL collaborators. Oral fluids will also be collected by a University of Colorado MPH student from pigs on the “Evaluation of killed *Mycobacterium bovis* vaccine to protect feral swine from bovine tuberculosis” via ropes and swine apples. The student is dependent 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.

**Commented [LEA-A23]:** Need to explain what swine apples are

**Commented [LEA-A24]:** Need to add when



~~Locations: Savannah River Field Station, GA and CSU. Collaborations established via email communication.~~ UF and Savannah River collaborators are on a presentation to be given at the International Wildlife Disease Association Conference in Chiapas, Mexico in July, 2017.

~~Project: "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)

Commented [LEA-A25]: Missing parts for this one

Commented [LEA-A26]: Are these actually at our facility or on city land?

~~Project: "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. (b)(3)

~~Project: "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.

~~Status:~~ 18 bison are going through the quarantine protocol. The youngest animals that need to complete quarantine per agreements were born in 2017. This means this project will end in 2020 or 2021. We will have to develop a plan to ensure these animals complete quarantine ~~or reach~~ agreement on disposing of these bison.

~~Agreement:~~

Commented [LEA-A27]: Are these actually at our facility or on city land?

Commented [NP-A28R27]: They are at our facility

Commented [LEA-A29]: I thought there was a signed agreement on this project.

Commented [NP-A30R29]: Need to verify which agreements to put here

~~Project: Development of safe and effective immobilization protocols for wild swine.~~

~~Collaborators: WS/NWRC, Colorado Parks and Wildlife; Texas A&M University; Wildlife Pharmaceuticals~~

~~Location:~~

~~Status:~~ Study will be completed by September 30, 2017.

## 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 four applicable agreements. (2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC, OA 1858 Protocol). This study is being conducted under NWRC approved Protocol 1858, and protocol approval from the Bison Quarantine Facility IACUC. The Interagency Agreement and the EA specified the disposition options for the seropositive and seronegative bison in the studies. They include

Commented [LEA-A31]: Can you clarify what the four are?

Commented [LEA-A32]: Can you send this protocol?

Commented [LEA-A33]: Please add the disposition options

## Cooperative Agreements Status

Commented [LEA-A34]: Add info on status of agreements.

## DISPOSITION OF ANIMALS

WiLDIT Wildlife Research Facility, Fort Collins, CO:

### ELK

~~All brucellosis seropositive elk have previously been euthanized.~~ Attempts will be made to place the remaining 34 brucellosis seronegative elk at another research facility (~~NEAH~~NADC, Colorado Parks and Wildlife, CSU, or Wyoming Game and Fish Department). ~~There are~~We have no prior agreements associated with the disposition of these animals. ~~Since they are within a CWD endemic area, this may~~ The one issue that may limit their ability to be moved, ~~is they are within a CWD endemic area.~~

Commented [LEA-A35]: Is this accurate the way I reworded it? Do you mean that this part of CO where we have our facility is considered endemic? What about where they were moved from?

Commented [NP-A36R35]: Yes, we are endemic. They would be alright to move to another facility in the endemic area such as Colorado or southern Wyoming. NADC would have to put them directly into BSL3 were they to accept them.

### BISON

Currently, in Fort Collins, 18 bison are going through the approved APHIS quarantine protocol. Disposition of animals (as per 2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC QA 1858 Protocol). We would like to discuss with CSU the potential continuation of the quarantine by CSU in CSU facilities. Quarantine of these animals would be completed in 2022 at the latest.

Commented [LEA-A37]: Provide when started and when would end.

Commented [NP-A38R37]: There are animals ranging from this year's calves to 3 years old. Minimum time to get through quarantine for a cow is 3.5 years but can be 4 5 years.

### SWINE

Two breeding herds exist: TX feral swine and HI feral swine. Both herds have 8-10 breeding sows and 2 boars. Because these animals have been trained to be handled, and the HI 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 Sept, 2017.

SPRS Bison Research Facility, Corwin Springs, MT

Don Herriot will take the lead in closing down this project according to the agreements in place.

Samples will be collected as indicated in the protocol

Commented [LEA-A39]: Is this accurate?

## ~~EQUIPMENT~~ PROPERTY DISPOSITION

WiLDIT personnel will ~~provide~~ gather their inventory records ~~and will work with~~ to Marj Swanson, CEAH, and Mary Waterbury, NVSL, for suitable disposition of equipment and supplies at the CO 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.

Commented [LEA-A40]: Do we have any property up at MT pens that should be on WiLDIT's property list vs. SPRS? I have sent email to Don to see what he thinks as well.

## OTHER LOGISTICS

#### Pens in MT – coordinating 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 FS and NPS may have interest in leasing two of the sites. One of the leases is funded through a cooperative agreement with the MT 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 CO

- There is no lease for the WiLDIT pens in CO that are on CSU property. There is a MOU with a sixty 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. CSU may elect to maintain the pens on their property or request that the ground be returned to its original state.

## NOTIFICATIONS

On acceptance of the plan, several notifications will take place.

- The VS Deputy Administrator will notify the state veterinarians in WY, MT, CO and TX of the disposition of the WiLDIT projects they have been involved with. The HI state veterinarian will be notified when it is determined if the feral swine from Hawaii are transferred to a collaborator.
- Collaborators will be notified by Dr. Jack Rhyan.
- The National Park Service will be notified by Drs. Jack Rhyan and Don Herriott
- Any notification to VS in a message from Dr. Jack Shere?
- \_\_\_\_\_

Commented [LEA-A41]: Has the state vet from TX been involved in project?

Commented [NP-A42R41]: Texas A and M Kingsville are involved and WS

Commented [LEA-A43]: Is this how to note this?

Commented [LEA-A44]: Are there other notifications?

Commented [LEA-A45]: Other notifications?



## IMPLEMENTATION PLAN TO DISBAND WiLDIT

### SUMMARY

This plan addresses the request from the Office of the Deputy Administrator for a plan to immediately ramp down the work of WiLDIT, ~~else~~evacuate the research facilities at the NWRC in Fort Collins and the pens at Corwin Springs, Montana 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 Understandings (MOU) in place with other entities regarding the project and disposition of the research animals is included. Personnel will be moved out of the WiLDIT group by the end of FY17. The disposition of all animals will be determined by June 30, 2017. In a few cases, dDiscussions with project collaborators are needed after approval of the implementation plan to determine if they would want to acquire the research animals. None of the animals that would be transferred would be positive for brucellosis. The seropositive and seronegative bison at the MT facilities would be disposed of or transferred per agreements with the National Park Service. Colorado State University would receive the sixty day termination notice provided for in the MOU regarding xxxx.

It has been determined that a 1010 package and Congressional notification are not required for this organizational change, however, a Civil Rights Impact Analysis is needed.

**Commented [MMP-A1]:** This is not a reasonable time frame since we have not even been cleared to talk to anyone about where they can be placed.

### PERSONNEL PLACEMENT

The four permanent employees currently assigned to the WiLDIT group will retain their grades and be reassigned pending approval from the Civil Rights Impact Analysis which is currently underway. The term employee is in the second year of a possible four-year position which expires March 6, 2018. There is one Saul T. Wilson student (b) (6) There are xxxx8 part-time students hired through CSU which will conclude employment by xxxxJuly 31, 2017.

- Dr. Jack Rhyan will be assigned to NVSL to lead 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. His duty station would remain in CO.
- Dr. Pauline Nol, Veterinary Medical Officer, and Matt McCollum, Wildlife Biologist, will be reassigned to CEAH to provide wildlife expertise to CEAH projects. They are meeting with the CEAH Director to determine the best organizational unit at CEAH to be reassigned to.
- Samantha Bruce, the Saul T. Wilson student, will transfer to CEAH. Since (b) (6)
- Karl Held, Animal Health Technician, will be assigned to the SPRS District 6 office in Lakewood, CO, with his duty station as his home.

**Commented [LEA-A2]:** Please fill in the two XXXX areas. Do we actually hire them or CSU does and then do we reimburse somehow?

- 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 (b) (6)

She is meeting with the CEAH Director to determine where her skills would best be used. WS may have at least partial salary funding to allow completion of a feral swine collaborative project.

## RESEARCH PROJECTS WRAP-UP

### Colorado Projects

**Project:** 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 brucellosis negative bison and to be completed in November 2017. APHIS does not have ownership of these animals. We will visit with the owners of the animals (Nature Conservancy) and NWRC about ending this project early. If not, Jack Rhyan will discuss transfer of those duties with NWRC.

**Agreements:** Evaluation of GonaCon (See document Protocol QA1923). This agreement requires tissues be taken for histopathology. Jack Rhyan will do the histopathology examination. Writing this project up will be done jointly with NWRC and Jack Rhyan. This study is being conducted under NWRC approved Protocol QA1923.

**Project:** "Brucellosis infection and transmission dynamics in elk."

**Collaborators:** WYG&F, ARS, CSU

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Project was halted in February of 2017 prior to mixing seronegative and seropositive elk that were collected in 2017, and all seropositive elk were removed by April, 2017.

Collaborators were notified in February and March of 2017. This project did not have any agreements with collaborators. A CSU approved IACUC protocol was in place (#14-4956A).

Collection permits were in place with Wyoming Game and Fish (#33-1040-2017; #33-1041-2017) and Department and Colorado Parks and Wildlife (#17TR2152)

**Project:** "Development of DryDart technology to deliver brucellosis vaccine to bison"

**Collaborators:** ARS/NADC

**Location:** ARS/NADC WiLDIT Wildlife Research Facility, Fort Collins, CO

**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.

**Commented [LEA-A3]:** Any type of graphic we can do on the timeline for projects in addition to the text? Maybe a CEAH technical writer can help us Mon.

**Commented [LEA-A4]:** Is this the Nature Conservancy Ranch?

**Commented [LEA-A5]:** How did we know they were seronegative? Did they go through quarantine procedures somewhere?

**Commented [NP-A6R5]:** These animal are from a brucellosis negative herd. For this study their status is irrelevant so no need for this statement

**Commented [LEA-A7]:** Is there any impact on study results by ending before end of Sept vs. November?

**Commented [NP-A8R7]:** These animals are only rounded up in November of each year. If they want to keep the animals then there is no impact. If we have to arrange for them to be euthanized then that will be problematic to do so before November

**Commented [LEA-A9]:** Where did the bison for this study come from?

**Commented [NP-A10R9]:** They are animals on the premises and are owned by the Nature Conservancy

**Commented [LEA-A11]:** Do you have this protocol in case asked for it?

**Commented [NP-A12R11]:** Now in document

**Commented [LEA-A13]:** Is this accurate?

**Commented [NP-A14R13]:** All elk collected from WY in 2017 were in same pen, and it turned out they were a mixture of positive and negatives. And Non pregnant seropositive elk from 2016 were mixed with seronegatives. I suggest leaving this statement out because it is misleading and not useful in this document.

**Commented [LEA-A15]:** Do we need a statement about how determined status so might have mixed seropos with seroneg?

**Commented [NP-A16R15]:** Is this necessary to even discuss here? I suggest leave it out because it is confusing and this issue has been resolved.

**Formatted:** Font: (Default) Times New Roman, 12 pt

**Commented [LEA-A17]:** Is the study at NADC?

**Commented [NP-A18R17]:** Yes as stated in document

An additional pilot study to evaluate the use of DryDarts to deliver immobilizing agents to animals ~~will be~~ is being conducted at the WiLDIT facility in Fort Collins in summer 2017 with completion before the end of ~~but will be complete by the end of~~ September 2017.

**Commented [LEA-A19]:** What type of animals? Anything seropositive? Is it in Fort Collins?

**Commented [NP-A20R19]:** I'll have to touch base with Jack on this

**~~Project:~~ "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:** There is an MOU that requires a 60 day notice for termination. This project used bison owned by APHIS that will now be no longer available for use by CSU. These bison are also under the four agreements applicable to YNP bison.

**~~Project:~~ 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 TB-free herd. At this time, these animals will no longer be the primary responsibility of WiLDIT. A master's student is dependent on having these animals available for sampling in the BSL-3 for her oral fluids project (Two Agreements: Alfano, Marialexia-Learning Plan; Alfano, Marialexia-Placement Agreement"). Animals will be necropsied November/December 2017. Disposition of pigs is 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).

**Commented [LEA-A21]:** Can we put something on disease status of these animals? Are they brucellosis and TB negative?

**Commented [NP-A22R21]:** These animals are brucellosis and TB negative based on disease free parentage. The herd is disease free

**~~Project:~~ "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:** Current cooperative agreement with Rovira i Virgili University will be completed and closed out ~~by XXXX~~ 14 August, 2017 and manuscript(s) prepared and submitted for publication based on data collected from previous projects. Intended sample collections from current projects will be taken over by NWRC or will not take place.

**Commented [LEA-A23]:** Can we put date?

**~~Project:~~ "Development of oral fluid collection studies for feral swine"**

**Collaborators:** CSU, VS-SPRS; University of Florida; University of Georgia.

**Locations:** Savannah River Field Station, GA 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 University of GA and UFL collaborators. Oral fluids will also be collected by a University of Colorado MPH student from pigs on the "Evaluation of killed *Mycobacterium bovis* vaccine to protect feral swine from bovine tuberculosis" via ropes and swine apples. The student is dependent 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.

**Commented [LEA-A24]:** Need to explain what swine apples are

**Commented [LEA-A25]:** Need to add when



~~Location: Savannah River Field Station, GA and CSU. Collaborations established via email communication.~~ UF and Savannah River collaborators are on a presentation to be given at the International Wildlife Disease Association Conference in Chiapas, Mexico in July, 2017.

~~Project: "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)~~

Commented [LEA-A26]: Missing parts for this one

Commented [LEA-A27]: Are these actually at our facility or on city land?

~~Project: "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. (b)(3)~~

~~Project: "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.~~

~~Status: 18-20 bison are going through the APHIS approved quarantine protocol. The youngest animals that need to complete quarantine per agreements were born in 2017. This means this project will end in 2020 or 2021. We will have to develop a plan to ensure these animals complete quarantine or reach agreement on disposing transferring ownership of these bison.~~

~~Agreement:~~

Commented [LEA-A28]: Are these actually at our facility or on city land?

Commented [NP-A29R28]: They are at our facility

Commented [MMP-A30]: Discussions in the past with Dr. Keith Roehr, indicate that as long as the standards are met, he does not have an issue with CSU conducting quarantine.

Commented [MMP-A31]: The two youngsters will need to have their mothers survive until they can be weaned. After weaning, the mothers can be sent to slaughter. (they will be 6 months old in January)

~~Project: Development of safe and effective immobilization protocols for wild swine.~~

~~Collaborators: WS/NWRC, Colorado Parks and Wildlife; Texas A&M University; Wildlife Pharmaceuticals~~

~~Location:~~

~~Status: Study will be completed by September 30, 2017.~~

Commented [LEA-A32]: I thought there was a signed agreement on this project.

Commented [NP-A33R32]: Need to verify which agreements to put here

## 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 four applicable agreements. (2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC. QA 1858 Protocol). This study is being conducted under NWRC approved Protocol 1858 and protocol approval from the Bison Quarantine Facility IACUC. The Interagency Agreement and the EA specified the disposition options for the seropositive and seronegative bison in the studies. They include

Commented [LEA-A34]: Can you clarify what the four are?

Commented [LEA-A35]: Can you send this protocol?

Commented [LEA-A36]: Please add the disposition options

## Cooperative Agreements Status

**Commented [LEA-A37]:** Add info on status of agreements.

**Commented [MMP-A38]:** This needs to be addressed by Don and his team. They are the ones who are best versed in the intricacies of these projects.

## DISPOSITION OF ANIMALS

WiLDIT Wildlife Research Facility, Fort Collins, CO:

### ELK

~~All brucellosis seropositive elk have previously been euthanized.~~ Attempts will be made to place the remaining 34 brucellosis seronegative elk at another research facility (~~NEAH~~ NADC, Colorado Parks and Wildlife, CSU, or Wyoming Game and Fish Department). ~~There are~~ We have no prior agreements associated with the disposition of these animals. ~~Since they are within a CWD endemic area, this may~~ The one issue that may limit their ability to be moved ~~is they are within a CWD endemic area.~~

**Commented [LEA-A39]:** Is this accurate the way I reworded it? Do you mean that this part of CO where we have our facility is considered endemic? What about where they were moved from?

**Commented [NP-A40R39]:** Yes, we are endemic. They would be alright to move to another facility in the endemic area such as Colorado or southern Wyoming. NADC would have to put them directly into BSL3 were they to accept them.

### BISON

Currently, in Fort Collins, ~~18-20~~ bison are going through the approved APHIS quarantine protocol. Disposition of animals (as per 2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC QA 1858 Protocol). We would like to discuss with CSU the potential continuation of the quarantine by CSU in CSU facilities. Quarantine of these animals would be completed in 2022, at the latest.

**Commented [LEA-A41]:** Provide when started and when would end.

**Commented [NP-A42R41]:** There are animals ranging from this year's calves to 3 years old. Minimum time to get through quarantine for a cow is 3.5 years but can be 4-5 years.

### SWINE

Two breeding herds exist: TX feral swine and HI feral swine. Both herds have 8-10 breeding sows and 2 boars. Because these animals have been trained to be handled, and the HI 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 Sept, 2017.

SPRS Bison Research Facility, Corwin Springs, MT

Don Herriot will take the lead in closing down this project according to the agreements in place. Samples will be collected as indicated in the [protocol]

**Commented [LEA-A43]:** Is this accurate?

## EQUIPMENT-PROPERTY DISPOSITION

WiLDIT personnel will provide ~~gather~~ their inventory records ~~and will work with~~ to Marj Swanson, CEAH, and Mary Waterbury, NVSL, for suitable disposition of equipment and supplies at the CO 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.

**Commented [LEA-A44]:** Do we have any property up at MT pens that should be on WiLDIT's property list vs. SPRS? I have sent email to Don to see what he thinks as well.

## OTHER LOGISTICS

#### Pens in MT – coordinating 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 FS and NPS may have interest in leasing two of the sites. One of the leases is funded through a cooperative agreement with the MT 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 CO

- There is no lease for the WiLDIT pens in CO that are on CSU property. There is a MOU with a sixty 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 their property or request that the ground be returned to its original state.

## NOTIFICATIONS

On acceptance of the plan, several notifications will take place.

- The VS Deputy Administrator will notify the state veterinarians in WY, MT, CO and TX of the disposition of the WiLDIT projects they have been involved with. The HI state veterinarian will be notified when it is determined if the feral swine from Hawaii are transferred to a collaborator.
- Collaborators will be notified by Dr. Jack Rhyan.
- The National Park Service will be notified by Drs. Jack Rhyan and Don Herriott
- Any notification to VS in a message from Dr. Jack Shere?
- 

**Commented [LEA-A45]:** Has the state vet from TX been involved in project?

**Commented [NP-A46R45]:** Texas A and M Kingsville are involved and WS

**Commented [LEA-A47]:** Is this how to note this?

**Commented [LEA-A48]:** Are there other notifications?

**Commented [LEA-A49]:** Other notifications?



## IMPLEMENTATION PLAN TO DISBAND WILDIT

### SUMMARY

This plan addresses the request from the Office of the Deputy Administrator for a plan to immediately ramp down the work of WildIT, close the research facilities at the NWRC in Fort Collins and the pens at Corwin Springs, Montana 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 Understandings (MOU) in place with other entities regarding the project and disposition of the research animals is included. Personnel will be moved out of the WildIT group by the end of FY17. The disposition of all animals will be determined by June 30, 2017. In a few cases, discussions with project collaborators are needed after approval of the implementation plan to determine if they would want to acquire the research animals. None of the animals that would be transferred would be positive for brucellosis. The seropositive and seronegative bison at the MT facilities would be disposed of per agreements with the National Park Service. Colorado State University would receive the sixty day termination notice provided for in the MOU regarding xxxx.

It has been determined that a 1010 package and Congressional notification are not required for this organizational change, however, a Civil Rights Impact Analysis is needed.

### PERSONNEL PLACEMENT

The four permanent employees currently assigned to the WildIT group will retain their grades and be reassigned pending approval from the Civil Rights Impact Analysis which is currently underway. The term employee is in the second year of a possible four-year position which expires March 6, 2018. There is one Saul T. Wilson student (b) (6). There are xxxx part-time students hired through CSU which will conclude employment by XXXX.

- Dr. Jack Rhyan will be assigned to NVSL to lead 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. His duty station would remain in CO.
- Dr. Pauline Nol, Veterinary Medical Officer, and Matt McCollum, Wildlife Biologist, will be reassigned to CEAH to provide wildlife expertise to CEAH projects. They are meeting with the CEAH Director to determine the best organizational unit at CEAH to be reassigned to.
- Samantha Bruce, the Saul T. Wilson student, will transfer to CEAH. Since (b) (6)
- Karl Held, Animal Health Technician, will be assigned to the SPRS District 6 office in Lakewood, CO, with his duty station as his home.

**Commented [LEA-A1]:** Please fill in the two XXXX areas. Do we actually hire them or CSU does and then do we reimburse somehow?

- 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 (b) (6)

She is meeting with the CEAH Director to determine where her skills would best be used. WS may have at least partial salary funding to allow completion of a feral swine collaborative project.

## RESEARCH PROJECTS WRAP-UP

### Colorado Projects

**Project:** Evaluation of duration of infertility produced by GonaCon, an immuno-contraceptive vaccine, in bison.

**Collaborators:** The Nature Conservancy, WS/NWRC

**Location:** (b) (6) /The Nature Conservancy in southern Colorado

**Status:** Started in 2011 with brucellosis negative bison and to be completed in November 2017. APHIS does not have ownership of these animals. We will visit with the owners of the animals (Nature Conservancy) and NWRC about ending this project early. If not, Jack Rhyan will discuss transfer of those duties with NWRC.

**Agreements:** Evaluation of GonaCon (See document Protocol QA1923). This agreement requires tissues be taken for histopathology. Jack Rhyan will do the histopathology examination. Writing this project up will be done jointly with NWRC and Jack Rhyan. This study is being conducted under NWRC approved Protocol QA1923.

**Project:** "Brucellosis infection and transmission dynamics in elk"

**Collaborators:** WYG&F, ARS, CSU

**Location:** WiLDIT Wildlife Research Facility, Fort Collins, CO

**Status:** Project was halted in February of 2017 prior to mixing seronegative and seropositive elk, and all seropositive elk were removed by April 2017. Collaborators were notified in February and March of 2017. This project did not have any agreements with collaborators. Collection permits were in place with Wyoming Game and Fish (#33-1040-2017; #33-1041-2017) and Department and Colorado Parks and Wildlife (#17TR2152)

**Project:** "Development of DryDart technology to deliver brucellosis vaccine to bison"

**Collaborators:** ARS/NADC

**Location:** ARS/NADC, WiLDIT Wildlife Research Facility, Fort Collins, CO

**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.

An additional pilot study to evaluate the use of DryDarts to deliver immobilizing agents to animals will be being conducted at the WiLDIT facility in Fort Collins in summer 2017 with completion before the end of but will be complete by the end of September 2017.

**Commented [LEA-A2]:** Any type of graphic we can do on the timeline for projects in addition to the text? Maybe a CEAH technical writer can help us Mon.

**Commented [LEA-A3]:** Is this the Nature Conservancy Ranch?

**Commented [LEA-A4]:** How did we know they were seronegative? Did they go through quarantine procedures somewhere?

**Commented [LEA-A5]:** Is there any impact on study results by ending before end of Sept vs. November?

**Commented [LEA-A6]:** Where did the bison for this study come from?

**Commented [LEA-A7]:** Do you have this protocol in case asked for it?

**Commented [LEA-A8]:** Is this accurate?

**Commented [LEA-A9]:** Do we need a statement about how determined status so might have mixed seropos with seroneg?

**Commented [LEA-A10]:** Is the study at NADC?

**Commented [LEA-A11]:** What type of animals? Anything seropositive? Is it in Fort Collins?

**Project:** “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:** There is an MOU that requires a 60 day notice for termination. This project used bison owned by APHIS that will now be no longer available for use by CSU. These bison are also under the four agreements applicable to YNP bison.

**Project:** 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. At this time, these animals will no longer be the primary responsibility of WILDIT. A master's student is dependent on having these animals available for sampling in the BSL-3 for her oral fluids project (Two Agreements: Alfano, Marialexia-Learning Plan; Alfano, Marialexia-Placement Agreement). Animals will be necropsied November/December 2017. Disposition of pigs is incineration. Tissues will be collected and cultured at NVSL and histopathology read by Jack Rhyon 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).

**Commented [LEA-A12]:** Can we put something on disease status of these animals? Are they brucellosis and TB negative?

**Project:** “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:** Current cooperative agreement with Rovira i Virgili University will be completed and closed out by XXXX and manuscript(s) prepared and submitted for publication based on data collected from previous projects. Intended sample collections from current projects will be taken over by NWRC or will not take place.

**Commented [LEA-A13]:** Can we put date?

**Project:** “Development of oral fluid collection studies for feral swine”

**Collaborators:** CSU, VS-SPRS; University of Florida; University of Georgia.

**Locations:** Savannah River Field Station, GA and CSU.

**Status:** Oral fluids are currently being collected from feral swine in the field via swine apples deployed by University of GA and UFL collaborators. Oral fluids will also be collected by a University of Colorado MPH student from pigs on the “Evaluation of killed *Mycobacterium bovis* vaccine to protect feral swine from bovine tuberculosis” via ropes and swine apples. The student is dependent 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. Locations: Savannah River Field Station, GA and CSU. Collaborations established via email communication. UF and Savannah River collaborators are on a presentation to be given at the International Wildlife Disease Association Conference in Chiapas, Mexico in July, 2017.

**Commented [LEA-A14]:** Need to explain what swine apples are

**Commented [LEA-A15]:** Need to add when

**Project:** “Development of oral fluid collection studies for elk”



**Collaborators:**

**Location:**

**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)

Commented [LEA-A16]: Missing parts for this one

**Project:** "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. (b)(3)

**Project:** "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.

**Status:** 18 bison are going through the quarantine protocol. The youngest animals that need to complete quarantine per agreements were born in 2017. This means this project will end in 2020 or 2021. We will have to develop a plan to ensure these animals complete quarantine or reach agreement on disposing of these bison.

Commented [LEA-A17]: Are these actually at our facility or on city land?

**Agreement:**

Commented [LEA-A18]: I thought there was a signed agreement on this project.

**Project:** Development of safe and effective immobilization protocols for wild swine.

**Collaborators:** WS/NWRC, Colorado Parks and Wildlife; Texas A&M University; Wildlife Pharmaceuticals

**Location:**

**Status:** Study will be completed by September 30, 2017.

**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 four applicable agreements. (2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC, QA 1858 Protocol). This study is being conducted under NWRC approved Protocol 1858 and protocol approval from the Bison Quarantine Facility IACUC. The Interagency Agreement and the EA specified the disposition options for the seropositive and seronegative bison in the studies. They include:

Commented [LEA-A19]: Can you clarify what the four are?

Commented [LEA-A20]: Can you send this protocol?

Commented [LEA-A21]: Please add the disposition options

Commented [LEA-A22]: Add info on status of agreements.

Cooperative Agreements Status

**DISPOSITION OF ANIMALS**

## WiLDIT Wildlife Research Facility, Fort Collins, CO:

### ELK

All brucellosis seropositive elk have previously been euthanized. Attempts will be made to place the remaining 34 brucellosis seronegative elk at another research facility (~~NCAH~~NADC, Colorado Parks and Wildlife, CSU, or Wyoming Game and Fish Department). There are ~~We~~ have no prior agreements associated with the disposition of these animals. Since they are within a CWD endemic area, this may ~~The one issue that may~~ limit their ability to be moved ~~is they are within a CWD endemic area.~~

**Commented [LEA-A23]:** Is this accurate the way I reworded it? Do you mean that this part of CO where we have our facility is considered endemic? What about where they were moved from?

### BISON

Currently, in Fort Collins, 18 bison are going through the approved APHIS quarantine protocol. Disposition of animals (as per 2013 Interagency Agreement with Yellowstone National Park, 2012 Environmental Assessment Evaluation of GonaCon in Bison, and IACUC QA 1858 Protocol). We would like to discuss with CSU the potential continuation of the quarantine by CSU in CSU facilities.

**Commented [LEA-A24]:** Provide when started and when would end.

### SWINE

Two breeding herds exist: TX feral swine and HI feral swine. Both herds have 8-10 breeding sows and 2 boars. Because these animals have been trained to be handled, and the HI 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 Sept, 2017.

## SPRS Bison Research Facility, Corwin Springs, MT

Don Herriot will take the lead in closing down this project according to the agreements in place. Samples will be collected as indicated in the protocol.

**Commented [LEA-A25]:** Is this accurate?

## **EQUIPMENT PROPERTY DISPOSITION**

WiLDIT personnel will provide ~~gather~~ their inventory records and will work with ~~to~~ Marj Swanson, CEAH, and Mary Waterbury, NVSL, for suitable disposition of equipment and supplies at the CO 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.

**Commented [LEA-A26]:** Do we have any property up at MT pens that should be on WiLDIT's property list vs. SPRS? I have sent email to Don to see what he thinks as well.

## **OTHER LOGISTICS**

### Pens in MT – coordinating 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

FS and NPS may have interest in leasing two of the sites. One of the leases is funded through a cooperative agreement with the MT 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 CO

- There is no lease for the WiLDIT pens in CO that are on CSU property. There is a MOU with a sixty 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. CSU may elect to maintain the pens on their property or request that the ground be returned to its original state.

## NOTIFICATIONS

On acceptance of the plan, several notifications will take place.

- The VS Deputy Administrator will notify the state veterinarians in WY, MT, CO and TX of the disposition of the WiLDIT projects they have been involved with. The HI state veterinarian will be notified when it is determined if the feral swine from Hawaii are transferred to a collaborator.
- Collaborators will be notified by Dr. Jack Rhyan.
- The National Park Service will be notified by Drs. Jack Rhyan and Don Herriott
- Any notification to VS in a message from Dr. Jack Shere?
- 

Commented [LEA-A27]: Has the state vet from TX been involved in project?

Commented [LEA-A28]: Is this how to note this?

Commented [LEA-A29]: Are there other notifications?

Commented [LEA-A30]: Other notifications?



Proposed Project:

DRAFT

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

Investigators:

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

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

Background:

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

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission~~ the shedding of *B. abortus* which leads to persistence of the disease in infected populations.

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* ~~transmissions~~ shedding in a bison herd

Commented [MAW1]: Transmission is not addressed here. Change to "potential for transmission" or "shedding"

Commented [MAW2]: Generally, the concept is unique and interesting. Proposal requires some more critical review and statistical input prior to implementation.

Commented [MAW3]: Then is the sample size proposed sufficient to address this question?

Commented [MAW4]: See comment on title

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

Commented [MAW5]: How will you determine colonization? Is this once 3 yr post-vaccination? How much natural variation would you expect to see?

Commented [MAW6]: Excellent point. Study should continue after contraceptive effects have been lost.

3. Determine the effect of immune system stimulation via vaccination with GonaCon adjuvant on brucella titers and shedding.

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Indent at: 0.5"

#### Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites.
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison, looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Commented [MAW7]: Granted the sample size is too small to do this, but it is a very important point. What is the potential for confounding effects of local inflammatory response from the adjuvant. Without adjuvant vaccinated controls, this can't be determined.

Commented [MAW8]: This portion of the study is not fully developed enough to know whether this objective could be met

Commented [MAW9]: Power?

Commented [MAW10]: Power?

#### Research Plan:

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

Commented [MAW11]: How was this n selected? Will it allow adequate power based on expected outcomes?

Commented [MAW12]: What if the adjuvant has an effect? Lack of a group of adjuvant treated will result in confounding of results. Are results of "vaccination" from lack of reproduction or could they be confounded with immune stimulation? This design issue needs to be addressed before implementing this project.

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

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

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. (b)(3)

(b)(3)

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

(b)(3) 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. (b)(3)

(b)(3) satisfy the bison quarantine requirements as published in the UM&R will be used-made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. (b)(3)

#### Time line:

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

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

Commented [r13]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r14]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r15]: (b)(3)



**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 (b)(3) study animals, collect ova and semen for genetic conservation.

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

#### Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined, preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector
  1. Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting? I'm not sure if this is answering a behavioral question (do bison investigate aborted fetuses) or a disease question (how often do aborted fetuses initiate seroconversion).
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy whether they would have an infectious live birth or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

Commented [jgp16]: Would be helpful to have a table of response variables, estimated variation expected between treatment groups, and what question they are meant to answer.

Commented [jgp17]: (b)(3)

Formatted: Indent: Left: 1", No bullets or numbering

Commented [jgp18]: Does this mean that we expect all animals to be seronegative at the end of the study or that no new ones will have seroconverted. This seems like it is directly related to the first outcome.

Commented [MAW19]: Are they infected or seropositive?

Commented [MAW20]: How will you do this and is power sufficient?

Commented [jgp21]: How do you rule out in-utero transmission?

Commented [MAW22]: This, like many of the other outcomes, seem to be overstated a bit. Some insights will no doubt be gained, but would like to see more evidence that data will actually allow "determination" of these factors.

Commented [MAW23]: Power? Pretty small sample of bulls, particularly if not all are actively breeding.

**Proposed Project:**

**DRAFT**

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

**Investigators:**

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

USDA, APHIS, WS: Lowell Miller, Kathy Fagerstone

NPS : Margaret Wild and Jenny Powers (Have asked for their review and interest in representing NPS)

**Background:**

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

GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison (and sterility in some?). In limited studies, infertility has lasted 3 years or longer following a single injection of 1800µg or 3000µg. Its use has been proposed as a nonlethal method of decreasing the ~~prevalence of~~ brucellosis transmission probability in bison by preventing pregnancy and abortion or normal parturition during the active infection period and thereby preventing ~~transmission the shedding~~ of *B. abortus* which leads to persistence of the disease in infected populations.

**Major Objectives:**

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrus has on *B. abortus* colonization in naturally-infected female bison and determine whether a prolonged period of infertility allows the infection to run its course without resulting in infectious shedding events. It is important to see whether subsequent pregnancies following infertility would result in a non-infectious parturition.

**Minor Objectives:**

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison looking for differences between high vs. low titered dams.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

**Research Plan:**

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



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

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

Female bison will be identified with uniquely numbered ear tags and microchip identification. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015).

Bulls will be separated from the cows after breeding season, from December until July and subsequently relocated to commingle with the females from August to November. During the three abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored.

Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied

(b)(3) 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. (b)(3)

(b)(3) satisfy the bison quarantine requirements as published in the UM&R will be ~~used~~ made available for bison conservation programs away from Yellowstone National Park. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal. (b)(3)

#### Time line:

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

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

Commented [r1]: Provide an expected result to show that the effects of the vaccine should wear off by this time and the vaccinates should have calves in 2015. Other wise the females should be followed until they do have one or two calves to evaluate whether the contraception period allows an individual to complete the infection cycle and move in to a recovered state where they would not be likely transmission vectors.

Commented [r2]: These are valuable subjects to resolve whether they would in fact abort or not abort their first pregnancy and whether their titer would remain relatively low in the seropositive range during and following that first pregnancy.

Commented [r3]: (b)(3)

**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 (b)(3) study animals, collect ova and semen for genetic conservation.

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

**Expected outcomes:**

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in ~~reducing transmission of *B. abortus* in bison herds will be determined~~ preventing the shedding of *B. abortus* during the active infection period and whether the contraceptive actions would ultimately result in an individual that does not subsequently become a brucellosis transmission vector.
  - 1- Alternate Hypothesis: The contraceptive effects of GonaCon vaccine results in long term or permanent sterility.
2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined. What sort of effects do you expect to see? And what are the alternative outcomes if the expected results are not observed?
3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined. ?? The probability of sero-negative bison becoming infected because of exposure in a confined setting?
4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined. The probability that calves born to seropositive adult females would become seropositive through exposure to bacteria in milk consumed during nursing the dam. And... whether those seropositive bison would be less likely to have an abortion during their first pregnancy, whether they would have an infectious live birth, or whether their infection would resemble the same clinical response that infectious bison exposed as mature individuals 2 years old or older.
5. The risk of venereal transmission of *B. abortus* from seropositive adult females to seronegative bull bison will be examined. If the females of the pen are out of sync in their pregnancy cycle then late abortion events could be a complicating factor here.

Formatted: Indent: Left: 1", No bullets or numbering



United States  
Department of  
Agriculture

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease ~~shedding exposure to~~ *Brucella abortus*, the ~~causative agent of~~ bacteria that can cause brucellosis. APHIS plans to publish an environmental assessment concerning this project soon. We wanted to notify you of this potential project and are requesting your comments.

One significant way that brucellosis can be spread between infected and uninfected bison happens when infected animals give birth. The materials associated with giving birth contain *Brucella abortus*, and uninfected bison often become exposed to the infected material. We wanted to notify you of this potential project and are requesting your comments. The study that APHIS wants to conduct will investigate one way to decrease the potential for this exposure to take place by preventing infected bison from giving birth.

~~APHIS plans to publish an environmental assessment concerning this project soon.~~ Some of the animals that will be used in the study were captured last spring and the remainder will be captured in upcoming years. Blood samples will be collected from captured bison to test to see if there is evidence of brucellosis infection. Bison that test positive for the presence of brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. ~~Some of these animals were captured last spring and the remainder will be captured in upcoming years.~~ It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years.

In the proposed study, Hhalf of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the GonaCon® vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile from the vaccine, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept in separate areas during the study. Animals will be ~~maintained~~ cared for throughout the study and abortions and births will be monitored. Seronegative bison will be placed with the seropositive GonaCon®- ~~vaccinated~~ contracepted animals and with the seropositive non-~~contracepted~~ GonaCon®- ~~vaccinated~~ animals to evaluate transmission of brucellosis. Following each birthing event, all bison will be examined ~~for shedding of *Brucella* bacteria~~ to see if they have infected materials that are capable of transmitting *Brucella abortus* to other bison.



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



Tribal Leader  
Page 2

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, ~~Brucella negative~~Non infected animals that have tested negative for brucellosis that also meet the requirements for previously-established quarantine use will be placed on tribal or public lands.

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at [Terry.W.Clark@aphis.usda.gov](mailto:Terry.W.Clark@aphis.usda.gov) or by telephone at (919) 855-7167.

Sincerely,

John R. Clifford  
Deputy Administrator



United States  
Department of  
Agriculture

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease shedding of Brucella abortus, the causative agent of brucellosis. We wanted to notify you of this potential project and changes to our animal disease regulations and are requesting your comments.

APHIS plans to publish an environmental assessment concerning this project soon. final rule amending requirements for the interstate movement of livestock and poultry in title 9, Code of Federal Regulations (9 CFR). Specifically, we are considering changes to section 71.20, approval of livestock facilities, and section 71.21, tissue and blood testing at slaughter. The proposed changes, which are summarized below, will increase our ability to safeguard livestock and poultry through early detection and reduced spread of foreign, emerging, and domestic program diseases. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. Some of these animals were captured last spring and the remainder will be captured in upcoming years. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years. Half of the seropositive cows will be vaccinated with GonaCon®, an immun contraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept separate. Animals will be maintained and abortions and births monitored. Seronegative bison will be placed with the seropositive contracepted animals and with the seropositive non-contracepted animals to evaluate transmission. Following each parturition event, all bison will be examined for shedding of the organism.

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, animals that meet the requirements for quarantine will be placed on tribal or public lands.

#### **Approval of Livestock Facilities (9 CFR 71.20)**

Currently, to be an approved livestock market or to maintain approval, individuals legally responsible for the day to day operations of the livestock facility must meet certain conditions. Namely, they must sign an agreement entitled, "Approved Livestock Facility for Handling Livestock Pursuant to title 9 of the Code of Federal Regulations." In addition, they must keep records such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in, the facility. The records are required to be maintained for 2 years. The changes we are considering to the regulations would increase this recordkeeping requirement to 5 years.

Commented [DAR1]: if a proposed rule, say proposed



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

#### **Tissue and Blood Testing at Slaughter (9 CFR 71.21)**

Under 9 CFR 71.21, livestock or poultry moving interstate for slaughter or rendering can only be moved to a slaughtering or rendering establishment that has been approved and listed by the APHIS Administrator. For an establishment to be listed, the operator of the establishment must agree to a number of provisions, such as:

- Allowing APHIS and Food Safety and Inspection Service personnel, or APHIS contractors, access to the facility to take blood and tissue samples from animals at the facility
- Retaining individual identification of animals
- Providing office space with necessary furnishings, light, temperature control, and janitorial service

Under the current regulations, operators of slaughtering or rendering establishments are not required to sign an agreement, and there are no recordkeeping requirements for these establishments.

However, as amended in the draft final rule, operators of slaughtering and rendering establishments would be required to sign a listing agreement, if they move animals interstate. Owners or operators that do not move livestock or poultry interstate are not required to be listed. The agreement will show that operators agree to meet the requirements for listed slaughtering and rendering establishments. In the event of a disease outbreak, APHIS may need to collect samples at certain facilities. By having an agreement in place, it will be easier to detect and prevent the spread of foreign, emerging, or domestic animal diseases.

In addition, we are considering adding recordkeeping requirements. Owners and operators will be required to keep documents such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in the facility, for 5 years. Retaining these records, as well as those for approved livestock facilities, for 5 years will help us find potentially infected or exposed livestock or poultry more quickly and enable us to do a more in-depth traceback. Livestock owners will benefit from reductions in the time needed to find animals that have been exposed to disease, which may reduce the time needed for quarantines.

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project's potential changes. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at [Terry.W.Clark@aphis.usda.gov](mailto:Terry.W.Clark@aphis.usda.gov) or by telephone at (919) 855-7167.

Sincerely,

Commented [dcc2]: The current rule states that they must agree to allow us access, etc., in order to be listed by the Administrator so it is assumed they would agree in writing but the actual agreement is not in the current rule. I would change the wording to state that the agreement is not in the rule because they are already required to sign the agreement and many establishments have done so

Commented [DAR3]: True?

Commented [DAR4]: True?

Commented [dcc5]: Yes

Commented [DAR6]: If we have proposed these changes, do we want to enclose the proposed rule for additional info?

Tribal Leader  
Page 3

John R. Clifford  
Deputy Administrator





United States  
Department of  
Agriculture

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

Dear Tribal Leader:

The Animal and Plant Health Inspection Service (APHIS) values its developing partnerships with the Tribal Nations. Therefore, we are informing Tribal Nations about a potential project to evaluate the use of a contraceptive vaccine in bison to decrease shedding of *Brucella abortus*, the causative agent of brucellosis. We wanted to notify you of this potential project and changes to our animal disease regulations and are requesting your comments.

Formatted: Font: Italic

APHIS plans to publish an environmental assessment concerning this project soon, final rule amending requirements for the interstate movement of livestock and poultry in title 9, Code of Federal Regulations (9 CFR). Specifically, we are considering changes to section 71.20, approval of livestock facilities, and section 71.21, tissue and blood testing at slaughter. The proposed changes, which are summarized below, will increase our ability to safeguard livestock and poultry through early detection and reduced spread of foreign, emerging, and domestic program diseases. The project will involve the use of up to 72 seropositive bison cows, 24 seronegative bison cows and 8 seronegative bison bulls. Some of these animals were captured last spring and the remainder will be captured in upcoming years. It is anticipated that the project will begin in the spring of 2012 and continue for at least 6 years. Half of the seropositive cows will be vaccinated with GonaCon®, an immunocontraceptive vaccine currently approved for use in white-tailed deer. Experimental studies with the vaccine have shown that it is effective for approximately 3 years in bison following a single injection. If bison are rendered temporarily infertile, in theory, they should not transmit brucellosis to other bison. This study will examine that question. GonaCon®-vaccinated and non-vaccinated animals will be kept separate. Animals will be maintained and abortions and births monitored. Seronegative bison will be placed with the seropositive contracepted animals and with the seropositive non-contracepted animals to evaluate transmission. Following each parturitionbirthing event, all bison will be examined for shedding of the *Brucella* organismbacteria.

Formatted: Font: Italic

The project will be done at the double fenced facilities previously used for the bison quarantine feasibility study, located at Corwin Springs, Montana. At the end of the study, *Brucella-negative* animals that meet the requirements for quarantine will be placed on tribal or public lands.

Formatted: Font: Italic

#### Approval of Livestock Facilities (9 CFR 71.20)

Currently, to be an approved livestock market or to maintain approval, individuals legally responsible for the day-to-day operations of the livestock facility must meet certain conditions. Namely, they must sign an agreement entitled, "Approved Livestock Facility for Handling Livestock Pursuant to title 9 of the Code of Federal Regulations." In addition, they must keep records such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in, the



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

facility. The records are required to be maintained for 2 years. The changes we are considering to the regulations would increase this recordkeeping requirement to 5 years.

Commented [DAR1]: if a proposed rule, say proposed

#### **Tissue and Blood Testing at Slaughter (9 CFR 71.21)**

Under 9 CFR 71.21, livestock or poultry moving interstate for slaughter or rendering can only be moved to a slaughtering or rendering establishment that has been approved and listed by the APHIS Administrator. For an establishment to be listed, the operator of the establishment must agree to a number of provisions, such as:

- Allowing APHIS and Food Safety and Inspection Service personnel, or APHIS contractors, access to the facility to take blood and tissue samples from animals at the facility
- Retaining individual identification of animals
- Providing office space with necessary furnishings, light, temperature control, and janitorial service

Under the current regulations, operators of slaughtering or rendering establishments are not required to sign an agreement, and there are no recordkeeping requirements for these establishments.

Commented [dcc2]: The current rule states that they must agree to allow us access, etc., in order to be listed by the Administrator so it is assumed they would agree in writing but the actual agreement is not in the current rule. I would change the wording to state that the agreement is not in the rule because they are already required to sign the agreement and many establishments have done so

Commented [DAR3]: True?

However, as amended in the draft final rule, operators of slaughtering and rendering establishments would be required to sign a listing agreement, if they move animals interstate. Owners or operators that do not move livestock or poultry interstate are not required to be listed. The agreement will show that operators agree to meet the requirements for listed slaughtering and rendering establishments. In the event of a disease outbreak, APHIS may need to collect samples at certain facilities. By having an agreement in place, it will be easier to detect and prevent the spread of foreign, emerging, or domestic animal diseases.

In addition, we are considering adding recordkeeping requirements. Owners and operators will be required to keep documents such as weight tickets, sales slips, and records of origin, identification, and destination that relate to livestock that are in, or that have been in the facility, for 5 years. Retaining these records, as well as those for approved livestock facilities, for 5 years will help us find potentially infected or exposed livestock or poultry more quickly and enable us to do a more in-depth traceback. Livestock owners will benefit from reductions in the time needed to find animals that have been exposed to disease, which may reduce the time needed for quarantines.

Commented [DAR4]: True?

Commented [dcc5]: Yes

We hope this information is helpful to you. We look forward to continued collaboration with the Tribal Nations and welcome your comments regarding this project's potential changes. If you have any questions or would like to meet with us, please contact Dr. Terry Clark, Tribal Liaison, by email at [Terry.W.Clark@aphis.usda.gov](mailto:Terry.W.Clark@aphis.usda.gov) or by telephone at (919) 855-7167.

Commented [DAR6]: If we have proposed these changes, do we want to enclose the proposed rule for additional info?

Tribal Leader  
Page 3

Sincerely,

John R. Clifford  
Deputy Administrator

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

**Mission of the Wildlife Livestock Disease Investigations Team (WiLDIT):**  
*Developing science-based solutions to disease problems at the wildlife/livestock/human interface.*

## **Background**

In 1997, Veterinary Services (VS) organized the WiLDIT group. VS originally tasked this group to assist in Greater Yellowstone Area (GYA) animal health issues. In 1999, VS expanded WiLDIT's duties to engage in wildlife/domestic animal interface issues in general. WiLDIT oversees a captive wildlife research facility in Fort Collins, Colorado that houses wild and domestic hoof stock for controlled studies as needed which is located on Colorado State University (CSU) land. The WiLDIT group is officed at the APHIS Wildlife Services (WS) National Wildlife Research Center (NWRC).

The WiLDIT group has been managed by various VS positions since its formation. In 1997 it reported to the Western Regional Director, in 1999 to the VS Deputy's office, in 2000 to the National Animal Health Program, in 2007 to the Assistant Western Regional Director, in 2009 to the Western Regional Director, and then 2011 to the Assistant Western Regional Director. With the VS reorganization in late 2013, the group was placed in the Mycobacterium Brucella section of the Diagnostic Bacteriology Laboratory in the National Veterinary Services Laboratories.

## **Current WiLDIT Staff**

WiLDIT staff currently consists of two permanent Veterinary Medical Officers, two permanent Wildlife Biologists, one permanent Animal Health Technician, and one Saul T. Wilson scholar. Organizationally, WiLDIT is aligned under the National Veterinary Services Laboratories (NVSL). It reports to the Mycobacterium Brucella section head, Dr. Suelee Robbe-Austerman in the Diagnostic Bacteriology Laboratory.

Jack Rhyan, DVM, MS - Wildlife Pathologist (GS-0701/14)  
Pauline Nol, DVM, MS, PhD - Wildlife Epidemiologist (GS-0701/13)  
Matt McCollum, MS - Wildlife Biologist (GS-0486/11)  
Morgan Wehtje, MS - Wildlife Biologist (GS-0486/09)  
Karl Held - Animal Health Technician (GS-0704/05)  
Samantha Bruce - Saul T. Wilson Scholar (GS-0799/07)

## **Current Budget**

### **Appropriated VS Funds – Cattle Health**

Salaries/Benefits	\$500,918
Travel	\$ 5,000
Cooperative Agreements	\$ 50,000
Supplies	\$ 76,796
Miscellaneous	\$ 14,274
TOTAL	\$646,988

**Commented [NP-A1]:** Would like to up this to \$10K.  
Travel to Spain to do wild boar VOC work will cost approx.  
\$5K

### **Appropriated WS Funds – Feral Swine**

TBD – FY 16    \$240,000



Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

#### Agreements

There are four current cooperative agreements. WiLDIT does not currently receive direct outside funding.

Activity/Description	Cooperator	State	Begin Date	End Date	Agreement Amount
Detection of TB, Bruc, etc. in Swine	Fundacio URV in Tarragona, Spain	SPAIN	8/13/2016	8/14/2017	\$27,995.00
Inactivated Brucella abortus vaccine in mice	Colorado State University	CO	7/1/2016	6/30/2017	\$19,776.90
Molecular Detection of Mycobacterium bovis	Colorado State University	CO	8/15/2016	8/14/2017	\$13,063.03
TB bovis vaccine in feral swine	Colorado State University	CO	8/1/2016	7/31/2017	\$82,877.50
				<b>TOTAL</b>	<b>\$143,712.43</b>

#### Project Overviews and Accomplishments

##### Brucellosis

Brucellosis, *caused by Brucella abortus*, is a bacterial disease in cattle, elk, bison, pigs, and humans. In animals, brucellosis is transmitted when the infected mother has aborted and other animals come in contact with the aborted fetus. The WiLDIT group has made significant contributions to our understanding of brucellosis and its control.

- WiLDIT documented the pathology and epidemiology of brucellosis in *Yellowstone bison and contributed to the knowledge base in* several species, for example:
  - 1) WiLDIT confirmed brucellosis impacts the health and welfare of bison (there had been controversy regarding the transmission of the disease in bison with the National Park Service and others and work documented *B. abortus* as cause of bison abortions);
  - 2) WiLDIT documented *the pathology of the involvement of lungworms in the epidemiology of* brucellosis in marine *mammals*; and
  - 3) WiLDIT published work that elk are the primary source of infection for new cattle herds in the GYA. Others have since confirmed this work.
- The Bison Quarantine Feasibility Study demonstrated that by using the Uniform Methods & Rules (UM&R) protocol for bison quarantine, *Brucella*-negative adults could graduate from a quarantine process to be used to start or augment herds remote from Yellowstone National Park (YNP). This study utilized seronegative animals from YNP.

**Commented [LEA-A2]:** WiLDIT – please review and edit, I added to after our call.

**Formatted:** Font: Italic

**Formatted:** Font: Italic

**Commented [LEA-A3]:** WiLDIT - Anything to add on the significance of this?

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

- Dr. Jack Rhyan is a co-inventor of GonaCon™, an immunocontraceptive vaccine, that has been shown to greatly reduce shedding of the Brucella organism and transmission in bison. This allows for the potential to use non-lethal means to prevent disease transmission.
- DryDart is a patent-pending technology developed by WiLDIT that delivers lyophilized, powdered, or spray-dried vaccines and medications in a pelleted form and allows the use of a shotgun as a dart gun. This technology could be used to deliver brucellosis vaccines to bison but it has other applications such as remote delivery of other vaccines as well as medications such as antibiotics to wildlife and livestock.

Commented [LEA-A4]: WiLDIT – are there any others on the patent or should be recognized?

### *Foot-and-Mouth Disease*

Foot-and-mouth disease (FMD) is a virus that could devastate the U.S. agricultural economy if it entered this country. FMD affects cattle, sheep, and swine, but its effects on U.S. wildlife were unknown until WiLDIT conducted and published studies to better understand the potential role of wildlife.

- Working with NVSL-Foreign Animal Disease Diagnostic Laboratory, WiLDIT determined the 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. This has directly impacted the models on FMD disease spread and how APHIS will conduct surveillance in the face of an outbreak.

See also the list of publications spanning from 1994 to the present at the end of this paper.

## **Current Projects**

### *Brucellosis*

Evaluation of GonaCon™, a contraceptive vaccine, to stop transmission of brucellosis in bison. As noted, Brucellosis is transmitted between animals when the infected mother has aborted and other animals come in contact with the aborted fetus. Applying contraception to bison cows with brucellosis will stop transmission of the bacteria to other animals. This study utilized seropositive and negative bison from YNP.

- Projects:
  - Corwin Springs Bison Facility, MT.
    - Collaborators: APHIS/VS-SPRS (lead); WS/NWRC.
    - Animal numbers: 104 YNP bison.
    - Timeframe: Study to be completed in 2019.
    - See further information under the Approvals for Studies and Select Agent Discussions section.
  - Great Sand Dunes, CO.
    - Collaborators (WiLDIT has lead): WS/NWRC; The Nature Conservancy.
    - Animal numbers: 20 Great Sand Dunes National Park bison.
    - Timeframe: Study to be completed in 2017.
- Justification: The goal of this project is to provide a non-lethal method to eliminate brucellosis from GYA bison; benefits livestock industry, public, and federal and state agencies.
- Background on intended recent bison shipment from Corwin Springs facility to CO:

Commented [LEA-A5]: WiLDIT - Added this – anything to add

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

- Original shipment planned consisted of 14 seronegative males (calves and 1 yearling), 5 seropositive males (calves and yearlings), 11 seronegative females (calves and 1 yearling), and 3 seropositive females (calves and 1 yearling).
- Destined studies:
  1. Use of assisted reproductive techniques to salvage genetics from Brucella-exposed bison (CSU – lead)
  2. Monitoring pre-pubertal bison from infected herd for latent infection (“heifer syndrome”) (VS)
  3. RB51 vaccination using DryDart delivery. (VS)
  4. Development of volatile organic compound (VOC) detection as diagnostic tool for brucellosis. (VS)
- 5.4 Venereal transmission study. (VS)
- Timeframe for next window to bring bison to Fort Collins
  - Up to 16 animals (seropositive and negative) could be shipped down late spring 2017.

Brucellosis infection/transmission dynamics in elk. Brucellosis is spread when animals abort. In this project, WiLDIT staff places pregnant brucellosis-infected elk in a pen with elk that are negative for brucellosis. If a sick elk has an abortion, the infected fetus may transmit brucellosis to the other elk. With this study design, we can learn a lot about the natural disease in elk. WiLDIT also would plan to test brucellosis vaccines by putting brucellosis-infected elk in a pen with vaccinated elk. This study utilizes seropositive elk from the GYA and elk from commercial sources.

- Project:
  - WiLDIT Wildlife Research Pens, Fort Collins, CO.
  - Collaborators (WiLDIT has lead): Wyoming Game and Fish Department; Agricultural Research Service (ARS); CSU.
  - Animal numbers: 62 elk from Wyoming feed-grounds and commercial sources.
  - Timeframe: Expected completion 2019.
- Justification: Elk are a source of infection to livestock, yet there are many knowledge gaps about brucellosis in elk. Knowledge would benefit all in development of mitigations. A safe and valid model for vaccine testing in elk would be extremely valuable to develop a tool to control brucellosis in elk.

Development of DryDart technology to deliver brucellosis vaccine to bison. Through this project, WiLDIT is developing a safe, effective, durable, and practical method to deliver vaccines to in the form of DryDart. DryDart is a patent-pending biodegradable 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. Officials could use DryDart to vaccinate bison and other wildlife or livestock in the field against brucellosis, as well as other diseases.

- Project:
  - National Animal Disease Center (NADC), Ames, IA.
  - Collaborators: ARS, NADC, Bacterial Disease Unit (lead).
  - Animal numbers: 20 Corwin Springs study-origin bison.
  - Timeframe: Study to be completed 2018.
- Justification: Development of a method to remotely deliver vaccine to wildlife providing another tool to be used in the eradication of brucellosis. This technology has many more potential applications that would benefit livestock industry, public, and federal and state agencies.

Commented [NP.A6]: We plan to euthanized and necropsy all of our seropositive animals in the near future. Should we rewrite this section accordingly?

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

**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. This study utilizes foundation herd animals, seropositive and negative animals from YNP, as well as reproductive tissues from YNP animals sent to slaughter.

- Project:
  - WiLDIT Wildlife Research Facility, Fort Collins, CO.
  - Collaborators: CSU (lead); VS/SPRS.
  - Animal numbers: 13 commercial and zoo-origin bison; 5 YNP bison; 5 Idaho bison.
  - Timeframe: Study to be completed in 2020.
- Justification: Provides method to save genetics from brucellosis-infected YNP bison going to slaughter. Benefits all agencies.

**Development of volatile organic compound (smells) and oral fluid collection studies for feral swine.**

This project is looking at a way to detect disease in feral pigs without having to capture them. WiLDIT collects breath and feces of wild pigs to look at what unique smells are in pigs infected with different diseases. The pigs chew on a cloth ball and get it wet with saliva. WiLDIT collects the ball, squeeze out the oral fluids, and test it to see what diseases the pigs have.

- Projects:
  - Texas A&M University studies.
    - Collaborators (WiLDIT has lead): Texas A&M University; CSU; NWRC; Rovira i Virgili University, Spain.
    - Timeframe: Expected completion of studies in 2019.
  - Field studies. Collaborators (WiLDIT has lead): VS-SPRS; University of Florida; University of Georgia.
  - Timeframe: Study to be completed in 2017.
- Justification: This project could provide a cost-effective way to perform disease surveillance on wild pigs.

**Evaluation of killed preparations of *Brucella abortus* in mice.** In this project, WiLDIT can kill the bacteria and put it in a mist or a fine powder that a healthy animal could breathe in. This may be a way of getting an animal's immune system to protect them from the bacteria.

- Project:
  - Colorado State University, Fort Collins, CO.
  - Collaborators (WiLDIT has lead): CSU.
  - Number of animals: 125 mice of commercial origin.
  - Timeframe: Study to continue for next several years.
- Justification: This project will look at ways to develop an efficacious vaccine and delivery system for remote delivery to elk in the GYA. There is currently a crucial need to stop the spread of brucellosis in elk. This project benefits the GYA livestock industry and animal health agencies.

**Bison Conservation.** Established and augmented bison conservation herds using animals that were offspring of YNP bison. WiLDIT put all eligible animals through the quarantine protocol and released them on open space in Northern Colorado and Illinois. This study utilizes seronegative animals from the Corwin Springs study, our foundation herd, and CSU's assisted reproductive technology study.



Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

- Projects:
  - Established herd at Soapstone Prairie Natural Area/ Red Mountain Open Space.
    - Collaborators: CSU; City of Fort Collins; Larimer County.
  - Augmented herd at Midewin National Tallgrass Prairie.
    - Collaborator: U.S. Forest Service.
- Justification: In agreements with the National Park Service, WiLDIT uses stated animals would for conservation.

### ***Bovine Tuberculosis***

**Evaluation of killed *Mycobacterium bovis* and a vaccine to protect feral swine from bovine tuberculosis (TB).** 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. Feral swine at the border with Mexico are at risk of being infected with bovine tuberculosis due to their close proximity with infected cattle and possibly other animals in Mexico. Molokai Island, Hawaii has harbored feral swine with bovine tuberculosis which in the past has been transmitted to domestic cattle. A vaccine against bovine tuberculosis in feral swine would be an important tool for controlling and preventing movement of this disease.

- Project:
  - WiLDIT Wildlife Research Facility and CSU.
    - Collaborators (WiLDIT as lead): CSU; University Castilla la Mancha, Spain; Neiker Inc., Spain.
    - Animal numbers: Up to 120 Fort Collins origin feral swine.
    - Timeframe: Studies to be completed in 2020.
- Justification: The purpose of this project is for WiLDIT to develop a technique officials can use to remotely vaccinate wild swine for TB. Hawaii and other areas with infected wild swine populations currently need this technique.

**Development of volatile organic compound (VOCs) studies for detection of bovine tuberculosis in wild boar and feral swine and oral fluid collection studies for feral swine.** WiLDIT is evaluating the use of volatile organic compounds - or smells - in breath and feces to detect bovine tuberculosis in wild swine. This is a way we can detect animals with tuberculosis by collecting their breath and feces which is easier than having to perform skin testing or collect blood or other body samples. WiLDIT looks for volatile organic compounds that are unique in animals that have certain diseases. We can use this technology for remote detection of disease in wild populations. We can also apply the technology to domestic animals as a way for us to detect disease without having to handle the animals, such as in cattle (bovine tuberculosis in cattle has been researched in previous VOC studies).

- Project:
  - WiLDIT Wildlife Research Facility, Fort Collins, CO; CSU, Fort Collins, CO; Spain.
  - Collaborators (WiLDIT as lead): CSU; University Castilla la Mancha, Spain; Rovira i Virgili University.
  - Timeframe: Studies expected to be completed in 2019.
- Justification: The purpose of this project is for WiLDIT, and collaborators, to develop a method for animal health agencies to remotely monitor diseases in wildlife. Animal health agencies need this technique. The rope technology could provide a way to conduct surveillance on feral swine if there was an outbreak of a foreign animal disease such as FMD.

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

*Other Projects*

**Development of safe and effective immobilization protocols for wild swine.** It is difficult for animal health officials to use drugs to render pigs unconscious. Wild pigs are dangerous to handle. WiLDIT is 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.

- Project:
  - WiLDIT Wildlife Research Facility, Fort Collins, CO.
  - Collaborators (WiLDIT has lead): WS-NWRC; Colorado Parks and Wildlife; Texas A&M University; Wildlife Pharmaceuticals.
  - Animal numbers: Up to 25 feral swine of Fort Collins origin.
  - Timeframe: Study to be completed in 2017.
- Justification: This study will develop an effective and safe immobilization protocols for wild pigs.

**Approvals for Studies and Select Agent Discussions**

The Institutional Animal Care and Use Committees of Colorado State University, WS-National Wildlife Research Center, and the Bison Quarantine Facility Animal Care and Use Committee (ACUC) have all approved project protocols for the WiLDIT group as well as the ACUCs of the National Wildlife Research Center and the Bison Quarantine Facility depending on the study location. Seropositive animals have moved under the appropriate permits and as allowed by the Brucellosis UM&R and with the approvals of the involved state veterinarians. The studies that were to be done in elk to look at natural transmission from seropositive animals to seronegative animals received approvals to proceed from CSU Biosafety Office, the CO state veterinarian, and the CO Parks and Wildlife. The APHIS VS local office also reviewed the facility and the project plan. The paddock to house seropositive elk was double fenced surrounded by an alleyway and perimeter fence. Bird netting was placed over the paddock and attached to the most internal fence, which excluded small to mid-size predators, during potential abortion season.

Commented [LEA-A7]: WiLDIT – carefully review this and make sure is totally accurate, edit as needed

Commented [LEA-A8]: Anything to add on birdproofing or other changes made?

In recent discussions with the Agriculture Select Agent Services (AgSAS) on the natural transmission studies that have taken place and are underway at Corwin Springs, and the planned elk transmission study in CO, it became clear that while select agent regulations [9 CFR 121.4(d)(1)] provide for the exemption from the select agent requirements for “Any overlap select agent or toxin that is in its naturally occurring environment, provided that the agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.” transmission studies to replicate natural exposure would not be allowed in non-select agent registered space. In 2010, discussion with the WiLDIT group and the AgSAS confirmed that the policy regarding diagnostic samples from naturally infected animals outlined below in the April 2015 Guidance on the Inventory of Select Agents and Toxins was in place. In 2010 and 2011, conversations with AgSAS, VS Western Regional Directors Office, and the National Park Service took place regarding the then proposed immunocontraception (GonaCon™) study in bison. (b)(3)

Formatted: Space Before: 12 pt

As noted in the

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

Guidance below. (b)(3)

[Redacted text block]

Formatted: Font: Italic

Formatted: Font: Italic

The Federal Select Agent Program recommends that an entity document the means of ensuring that select agents are non-viable. Samples collected from animals that are presumed to have been naturally infected (i.e., not intentionally introduced) would not be considered select agent material and are not required to be handled as regulated material until the samples have been confirmed to contain select agent material. Diagnostic samples from which the presence of a select agent has been detected are subject to the select agent regulations; however, diagnostic samples from which only an antibody response to a select agent is detected are not considered select agent material. In addition, samples taken from animals experimentally infected with select agents are considered select agents and are subject to the select agent regulations, unless the absence of the agent can be demonstrated.

There have been no transmission studies done with bison at the WiLDIT facility in CO. (b)(3)

[Redacted text block]

Commented [LEA-A9]: Is this accurate? Any other transmission studies done that are not captured here?

[Redacted text block]

Commented [LEA-A10]: Is this an accurate description?

## Publications

Nol P., S. C. Olsen, J. C. Rhyan, N. Sriranganathan, M. P. McCollum, S. G. Hennager, A. A. Pavuk, P. J. Sprino, S. M. Boyle, R. J. Berrier, M. D. Salman. 2016. Vaccination of elk (*Cervus canadensis*) with *Brucella abortus* strain RB51 overexpressing superoxide dismutase and glycosyl-transferase genes does not induce adequate protection against experimental *Brucella abortus* challenge. *Frontiers in Cellular Infection and Microbiology*. 10;6:10.

Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. 2016. Kamath, P.L., J.T. Foster, K.P. Drees, G. Luikart, C. Quance, N.J. Anderson, P.R. Clarke, E.K. Cole, M.L. Drew, W.H. Edwards, J.C. Rhyan, J.J. Treanor, R.L. Wallen, P.J. White, S. Robbe-Austerman, and P.C. Cross. *Nature Communications* DOI: 10.1038/ncomms11448: 1-10.

Rhyan, J.C., D. Tyers, J. Zimmer, K. Lewandowski, S. Hennager, J. Young, R. Pappert, A. Panella, and

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

- O. Kosoy. 2015. Serologic survey of snowshoe hares (*Lepus americanus*) in the Greater Yellowstone Area for brucellosis, tularemia, and snowshoe hare virus. *Journal of Wildlife Diseases* 51: 769-773.
- Hobbs N.T., C. Geremia, J. Treanor, R. Wallen, P.J. White, M.B. Hooten, and J.C. Rhyan. 2015. State-space modeling to support management of brucellosis in the Yellowstone bison population. *Ecological Monographs* 85: 525-556.
- Clarke, R. R., R. K. Frey, J. C. Rhyan, M. P. McCollum, P. Nol, and K. Aune. 2014. Feasibility of quarantine procedures for bison (*Bison bison*) calves from Yellowstone National Park for conservation of brucellosis-free bison. *Journal of the American Veterinary Medical Association* 244(5): 588-591.
- Bayn, A, P. Nol, U. Tisch, J. Rhyan, C. K. Ellis, and H. Haick. 2013. Detection of volatile organic compounds in *Brucella abortus* -seropositive bison. *Analytical Chemistry* 85(22): 11146-11152.
- 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* 19(12): 1992-1995.
- McCollum, M., J. Rhyan, S. Coburn, D. Ewalt, C. Lahr, P. Nol, T. Keefe, C. Kimberling, and M. Salman. 2013. Clinical, culture, serology, and histopathology outcomes of bighorn sheep experimentally infected with *Brucella ovis*. *Journal of Wildlife Diseases* 49(4): 900-910.
- Rhyan, J.C., L. Miller, and K. A. Fagerstone. 2013. The use of contraception as a disease management tool in wildlife. *Journal of Zoo and Wildlife Medicine* 44(4S): S135-S137.
- Lambourn, D. M., M. Garner, D. Ewalt, S. Raverty, I. Sidor, S. J. Jeffries, J. Rhyan, and J. K. Gaydos. 2013. *Brucella pinnipedialis* infections in Pacific harbor seals (*Phoca vitulina richardsi*) from Washington State, USA. *Journal of Wildlife Diseases* 49(4): 802-815.
- Frey, R. K., R. Clarke, M. P. McCollum, P. Nol, K. R. Johnson, B. D. Thompson, J. M. Ramsey, N. J. Anderson, and J. C. Rhyan. 2013. Evaluation of bison (*Bison bison*) semen from Yellowstone National Park, Montana, USA, bulls for *Brucella abortus* shedding. *Journal of Wildlife Diseases* 49(3): 714-717.
- Uhrig, S.R., P. Nol, M. McCollum, M. Salman, and J. C. 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.
- Aune, K., J.C. Rhyan, R. Russell, T.J. Roffe, and B. Corso. 2011. Environmental persistence of *Brucella abortus* in the Greater Yellowstone Area. *Journal of Wildlife Management* 76: 253-261.
- Van Campen, H. and J. Rhyan. 2010. The role of wildlife in diseases of cattle. *Vet Clin Food Anim* 26: 147-161.
- Rhyan, J. C. and T.R. Spraker. 2010. Emergence of diseases from wildlife reservoirs. *Vet Pathol* 47:34-39



Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

- Schumaker, B.A., B. A. Corso, J. C. Rhyan, L. M. Philo, M. D. Salman, and I. A. Gardner. 2010. Evaluation of the fluorescence polarization assay for the detection of *Brucella abortus* antibodies in bison in a natural setting. *Comp Immunol Microbiol Inf Dis* 33:e119-e125.
- Nol, P., S. C. Olsen, and J. C. Rhyan. 2009. Experimental infection of Richardson's ground squirrels (*Spermophilus richardsonii*) with attenuated and virulent strains of *Brucella abortus*. *Journal of Wildlife Diseases*. 45: 189-195.
- Rhyan, J. C., K. Aune, T. Roffe, D. Ewalt, S. Hennager, T. Gidlewski, S. Olsen, and R. Clarke. 2009. Pathogenesis and epidemiology of brucellosis in Yellowstone bison: serologic and culture results from adult females and their progeny. *Journal of Wildlife Diseases*. 45: 729-739.
- Killian, G., T. Kreeger, J. 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.
- Rhyan, J.C. K. Aune, T. Roffe, D. Ewalt, S. Hennager, T. Gidlewski, S. Olsen, and R. Clarke. 2009. Pathogenesis and epidemiology of brucellosis in Yellowstone bison: Serologic and culture results from adult females and their progeny. *J Wildl Dis* 45:729-739.
- Fuller, J. A., R. A. Garrott, P. J. White, K. E. Aune, T. J. Roffe, and J. C. Rhyan. 2007. Reproduction and survival of Yellowstone bison. *The Journal of Wildlife Management* 71: 2365-2372.
- Olsen, S.C., J. Rhyan, T. Gidlewski, J. Goff, and W.C. Stoffregen. 2004. Safety of *Brucella abortus* Strain RB51 in Black Bears. *J Wildl Dis*. 2004 Jul;40(3):429-33.
- Miller, L. A., J. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. *J Wildl Dis*. 2004 Oct;40(4):725-30.
- Rhyan, J. C. and M. Drew. 2002. Contraception: a possible means of decreasing transmission of brucellosis in bison. In: Kreeger, T.J. (ed), *Brucellosis in Elk and Bison in the Greater Yellowstone Area*. Wyoming Game and Fish Department. Cheyenne, WY. 99-108.
- Cook, W. and J. Rhyan. 2002. Brucellosis vaccines and non-target species. In: Kreeger, T.J. (ed), *Brucellosis in Elk and Bison in the Greater Yellowstone Area*. Wyoming Game and Fish Department. Cheyenne, WY. 61-65.
- Rhyan, J. C., T. Gidlewski, T. J. Roffe, K. Aune, L. M. Philo, and D. R. Ewalt. 2001. Pathology of brucellosis in bison from Yellowstone National Park. *J Wildl Dis* 37: 101-109.
- Rhyan, J. C., T. Gidlewski, D. R. Ewalt, S. G. Hennager, D. M. Lambourne, S. C. Olsen. 2001. Seroconversion and abortion in cattle experimentally infected with *Brucella* sp. isolated from a Pacific harbor seal (*Phoca vitulina richardsi*). *J Vet Diagn Invest* 13: 379-382.
- Gidlewski, T., N. F. Cheville, J. C. Rhyan, L. D. Miller, and M. J. Gilsdorf. 2000. Experimental *Brucella abortus* induced abortion in a llama; pathologic effects. *Vet Pathol* 37: 77-82.
- Rhyan, J.C. 2000. Brucellosis in terrestrial wildlife and marine mammals. In: Brown, C. and C. Bolin (eds), *Emerging Infectious Diseases of Animals*. American Society for Microbiology Press. Washington, DC. 161-184.

Wildlife Livestock Disease Investigations Team  
Briefing Paper  
20 February 2017

Olsen, S.C., J. Rhyan, T. Gidlewski, and M. Palmer. 1999. Biosafety and immune responses following vaccination of adult bison bulls with *Brucella abortus* strain RB51. *American Journal of Veterinary Research* 60: 905-908.

Rhyan, J.C., K. Aune, T.J. Roffe, T. Gidlewski, D.R. Ewalt, and M. Philo. 1998. Lesions and sites of tissue localization of *Brucella abortus* in female bison from Yellowstone National Park: Preliminary results. (Appendix B) In: Cheville, N.F., D.R. McCollough, and L.R. Paulson. *Brucellosis in the Greater Yellowstone Area*. National Research Council, National Academy of Sciences, National Academy Press. Washington, D.C. pp. 177-180.

Ewalt, D.R., J.B. Payeur, J.C. Rhyan, and P.L. Geer. 1997. *Brucella suis* biovar 1 in naturally infected cattle: a bacteriological, serological, and histological study. *Journal of Veterinary Diagnostic Investigation* 9: 417-420.

Rhyan, J.C., K. Aune, D.R. Ewalt, J. Marquardt, J.W. Mertins, J.B. Payeur, D.A. Saari, P. Schladweiler, E.J. Shehan, and D. Worley. 1997. Survey of free-ranging elk from Wyoming and Montana for selected pathogens. *Journal of Wildlife Diseases* 33: 290-298.

Rhyan, J. C., S.D. Holland, T. Gidlewski, D.A. Saari, A.E. Jensen, D.R. Ewalt, S.G. Hennager, S.C. Olsen, and N.F. Cheville. 1997. Seminal vesiculitis and orchitis caused by *Brucella abortus* biovar 1 in young bison bulls from South Dakota. *Journal of Diagnostic Investigation* 9: 368-374.

Garner, M.M., D. M. Lambourn, S.J. Jeffries, P.B. Hall, J.C. Rhyan, D.R. Ewalt, L.M. Polzin, and N.F. Cheville. 1997. Evidence of *Brucella* infection in *Parafilaroides* lungworms in a Pacific harbor seal (*Phoca vitulina richardsi*). *Journal of Veterinary Diagnostic Investigation* 9: 298-303.

Rhyan, J.C., W.J. Quinn, L.L. Stackhouse, J.J. Henderson, D.R. Ewalt, J.B. Payeur, M. Johnson, and M. Meagher. 1994. Abortion caused by *Brucella abortus* biovar 1 in a free-ranging bison (*Bison bison*) from Yellowstone National Park. *Journal of Wildlife Diseases* 30: 445-4