USDA

Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of *Brucella abortus* in Bison in the Greater Yellowstone Area

Environmental Assessment, January 2012

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I. Introduction

A. Background

In Yellowstone National Park (YNP), wild and free-ranging bison (*Bison bison*) are critical parts of a fully-functioning ecosystem as well as being important to the identity of the park. The bison are a part of the esthetic, cultural, and natural environment of the YNP. YNP bison are chronically infected with brucellosis, a contagious disease that the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA/APHIS/VS) is striving to eliminate.

Brucellosis is a serious disease of livestock and wildlife that has significant animal and public health and international trade consequences. The disease is caused by bacteria of the genus *Brucella.* Brucellosis occurs primarily in cattle, bison, and swine; however, cervids, goats, sheep, and horses are also susceptible. In cattle and bison, the specific disease organism of concern is *Brucella abortus (B. abortus)*.

In its principal animal hosts, brucellosis causes loss of young through spontaneous abortion or birth of weak offspring, reduced milk production, and infertility. In cattle and bison, the disease localizes in certain lymph nodes, reproductive organs and/or the udder, causing spontaneous abortions in females and systemic effects in both male and female animals. Weight loss and lameness may also be associated with brucellosis infection.

The shedding[1] of *B. abortus* through the reproductive tract during an abortion or calving event may contribute to the transmission of infection to other animals that come in contact with the expelled bacteria now in the environment. Studies have shown that *Brucella* can persist on fetal tissues, vegetation and soil in YNP for as long as 81 days depending on environmental conditions (Aune et al., 2011). Spread of the disease occurs when the cattle and bison, which are social animals, sniff and lick a newborn calf, the afterbirth, and even an aborted fetus. This behavior provides an avenue for the disease to spread if *B. abortus* organisms are present. Additionally, *B. abortus* is present in the milk from infected females and can be transmitted to calves through suckling. There is no effective means of treating brucellosis in livestock or wildlife.

Studies investigating the prevalence of brucellosis in YNP bison have estimated that between 40% and 60% of YNP bison have been exposed to

[1] For purposes of the proposed study, "shedding" is to expel *B. abortus* from the body through the reproductive tract.

the disease. Further testing of animals that are seropositive[2] demonstrates that more than 40% of the seropositive animals are culture-positive, confirming actual infection with *B. abortus* (Meyer and Meagher, 1995; Cheville et al., 1998). In the areas outside the borders of YNP where livestock such as cattle are often raised, there is a concern that infected bison may transmit the disease to livestock if infected bison abort or calve.

Multiple Federal and state agencies[3] have participated in efforts to control the potential spread of brucellosis and conserve bison through the 2000 Interagency Bison Management Plan (IBMP) (MDoL and MFWP, 2000). In 1934, a federal brucellosis program was established as part of an effort to safeguard domestic livestock (See http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/ for additional information regarding USDA APHIS' brucellosis program).

Safeguarding measures, such as preventing, detecting, and eliminating animal diseases, help to maintain the U.S. cattle industry's national and international trade interests, ensure food safety, and protect public health. The efforts of the national brucellosis program have nearly eradicated brucellosis from domestic cattle and bison populations. As of July 2009, all 50 States had attained Class-Free (disease-free) status for brucellosis in domestic cattle and bison (USDA APHIS, 2010a). Currently, the last known reservoir of bovine brucellosis is in the wild bison and elk population in the Greater Yellowstone Area (GYA). Prevention of the spread of brucellosis between infected wildlife and livestock continues to be an issue of concern. The proposed study discussed in this environmental assessment (EA) is designed to investigate the feasibility of using an immunocontraceptive vaccine, GonaCon™, as a non-lethal management option to decrease the potential risk of disease transmission by brucellosis-infected bison.

In humans, Brucellosis is often referred to as undulant fever because it persists for several weeks or months and may get progressively worse if untreated. Humans are most commonly infected by consumption of unpasteurized dairy products produced from milk of infected animals, or they may become infected through direct contact with infected animal tissues such as aborted fetuses or reproductive materials. In humans, brucellosis initially causes flu-like symptoms that are treated with a rigorous course of antibiotics. In some isolated cases, the disease may develop into a variety of chronic conditions, including arthritis. Potential

[2] Bison that test positive on blood tests for brucellosis are referred to as being seropositive, and bison that do not test positive are referred to as being seronegative.

[3] U.S. Department of Interior National Park Service (NPS); U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS); U.S. Department of Agriculture Forest Service (FS); Montana Department of Livestock (MDoL); and Montana Fish, Wildlife and Parks (MFWP).

effects of the proposed study on humans will be discussed in the potential environmental impacts section.

GonaCon™ Immunocontraceptive Vaccine

GonaCon™ is a contraceptive vaccine that stimulates an immune response in a vaccinated animal by producing antibodies that bind to a gonadotropin-releasing hormone (GnRH). GnRH is a naturally occurring hormone that signals production of sex hormones such as estrogen, progesterone, and testosterone. The anti-GnRH antibodies interfere with the ability of GnRH to signal production of sex hormones, resulting in temporary infertility. As long as adequate levels of anti-GnRH antibodies are present in the vaccinated animal, sexual activity, breeding, and reproduction are extremely unlikely.

GonaCon™ is currently approved under the United States Environmental Protection Agency's (EPA's) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for use in female white-tailed deer as one tool to aid in reducing deer overpopulation (EPA Registration Number 56228-40). The immune response that causes temporary infertility in deer is accomplished with a single-shot vaccine. The length of time that a vaccinated female deer remains infertile depends on the individual animal, but some pen studies have shown that 4 out of 5 female deer remain infertile for 5 years (Miller et al., 2008a). Field studies have demonstrated lower rates of infertility ranging from 88% and 47% the first and second year after vaccination, respectively (Gionfriddo et al., 2009) to 67% and 43% the first and second year after vaccination, respectively (Gionfriddo et al., 2011a).

GonaCon™ is not currently registered for use in bison. However, USDA conducted a small pilot study of penned bison and found that none of the 6 females vaccinated with GonaCon™ became pregnant the first year after treatment (Miller et al., 2004). In 2011, APHIS received approval from EPA to use GonaCon™ in female bison in the confined experimental use scenario discussed in this EA. Should the proposed study discussed in this EA proceed, the data obtained from it could potentially be used to add to the required data set needed for EPA to register the GonaCon™ vaccine for use in bison. However, the purpose for registering GonaCon™ in bison would not be for reducing overpopulation. The intended purpose of using GonaCon™ in female bison would be to manage reproduction in bison known to be infected with brucellosis by inducing temporary infertility, thereby decreasing the potential for transmission of brucellosis through abortion and calving events.

B. Purpose of and Need for the Proposed Action

The purpose of the proposed action is to conduct a study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by preventing pregnancy, calving, and abortion, thereby preventing transmission of *B. abortus*. The major objectives of the proposed study are:

- To evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in *B. abortus*-infected female bison;
- To evaluate the effect on shedding by *B. abortus*-infected female bison that are rendered temporarily infertile by GonaCon™; and
- To evaluate the effect the infertility produced by GonaCon™ has on the long-term survivability of *B. abortus* in infected female bison.

Use of an effective immunocontraceptive such as GonaCon™ to prevent pregnancy and eliminate the potential for abortions by infected bison would break the cycle of transmission of brucellosis. If female bison known to be infected with *B. abortus* do not become pregnant, they would not abort. Exposure of non-infected animals to the infected tissues and fluids from aborted fetuses would therefore be reduced.

The need for the proposed study is to provide information that would be used to evaluate the use of GonaCon™ as a nonlethal method of decreasing or controlling the risk of transmission of *B. abortus* in the YNP bison population. Brucellosis is spread within the animal population primarily through contact with infected birthing tissues or aborted fetuses and through the milk of infected cows. If GonaCon™ can effectively render brucellosis-infected female bison temporarily infertile, the primary routes of disease transmission would be blocked. In combination with other appropriate disease mitigation activities, the use of GonaCon™ may be an effective tool to assist in eliminating brucellosis from the YNP bison herd over time.

USDA APHIS has determined that under the provisions of the National Environmental Policy Act (NEPA) (see 42 U.S.C. 4321 et seq.) and APHIS' National Environmental Policy Act (NEPA) implementing procedures (see 7 CFR Part 372), an EA should be prepared for these proposed actions. The availability of this EA and a 30-day comment period will be announced by publishing a notice on the APHIS brucellosis program website, the IBMP website and/or local newspapers. APHIS' decision maker for the actions described in this EA will take appropriate action after reviewing the EA, its associated analyses, public comments received, and other relevant responses and recommendations.

II. Proposed Action and Alternatives

A. No Action (the Current Situation)

The no action alternative would result in not conducting the proposed study. If the proposed study is not conducted, the utility of GonaCon™ as a non-lethal reproductive control option in bison cannot be determined. Additionally, if the use of GonaCon™ in bison is not investigated, information would not be known on whether temporary infertility induced by GonaCon™ is effective in decreasing the shedding of *B. abortus* and ultimately the transmission of brucellosis. Without the proposed study, use of the immunocontraception approach as a viable disease management tool for bison would not be evaluated, and could not be considered as a potential management tool.

B. Proposed Action

The proposed action is to conduct a multi-year study to evaluate the potential for use of GonaCon™, an immunocontraceptive vaccine, as a non-lethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy, thereby preventing abortions and risk of transmission of brucellosis to uninfected animals from contact with infected tissues and fluids from aborted fetuses.

The proposed study would include the following activities that are discussed in further detail below:

- Capturing bison in the late winter/spring of 2011, 2012, 2013, and possibly 2014;
- Transporting the captured bison by stock trailer to APHIS' bison facilities in Gardiner, Montana;
- Collecting and evaluating blood samples to determine brucellosis infection status at the beginning of the study and monitoring infection status at regular intervals throughout the study;
- Housing, caring for, and tagging (for identification purposes) animals in Gardiner, Montana facilities;
- Injecting one group of seropositive female bison with GonaCon™ beginning in the spring of 2012;
- Commingling uninfected bulls with females during breeding season, documenting breeding behavior, and testing for pregnancy for five calving seasons;
- Monitoring pregnant bison with transmitters and daily observing them for abortions, labor, and births;
- Collecting and testing blood, milk, and vaginal swabs from female bison that give birth to test for brucellosis infection status;

- Monitoring exposure to aborted fetuses by other bison and evaluating fetuses collected during the study; and
- Evaluating data collected from the study to determine whether GonaCon™ decreases the shedding of *B. abortus* in bison.

Bison for the proposed study would be acquired during the winter when they naturally exit YNP. The capture of bison would be conducted using methods currently in use for capturing bison according to the details of the IBMP operating procedures (IBMPOP, 2009). These procedures include hazing and/or using weed-free hay to move them to a capture facility. Approximately 104 adult bison would be used in the proposed study: 24 female bison that are seronegative for brucellosis; 72 female bison that test seropositive for brucellosis; and 8 male bison (bulls) that test seronegative for brucellosis. Female bison would be yearlings, two-, and three-years of age. If temporary chemical immobilization of any animal is needed, opioid narcotics and alpha-2-adrenergics would be used by study personnel qualified in the administration of such drugs. All bison used in the study would be identified with uniquely numbered ear tags and microchip identification.

The proposed study would take place on several double-fenced pastures at facilities in the Gardiner, Montana area: the Brogan Bison Facility in Corwin Springs (60 acres), the Slip ‘n Slide pasture (25 acres), and the Rigler pasture (32 acres), all of which are located north of Gardiner, Montana. All sites are within the GYA and along Highway 89. The Brogan Bison Facility, Rigler pasture, and Slip ‘n Slide pastures are currently leased by APHIS VS and Montana Fish, Wildlife and Parks and are used by APHIS VS for the bison quarantine feasibility study (MFWP, 2005). These facilities were specifically designed and erected to hold bison in a quarantine environment with hay and water as needed for an extended period of time.

The study design is as follows: In spring 2012, animals would be randomly selected to go into groups of 16 to18 seropositive cows, four to six seronegative cows, and two bulls. Two replicate test pastures would be established in 2013 and possibly 2014 if not enough animals are captured in 2013. After three to four weeks of acclimation in the test pastures, *B. abortus*-infected female bison in one of the pastures would receive GonaCon™ vaccine (containing 3,000 micrograms in 3 milliliters of an adjuvant) delivered into the muscle on each side of the neck. The sites of injection would be tattooed and observed for any injection reaction. Bison in the remaining pasture would not be vaccinated.

Bulls would be separated from the cows outside of the breeding season from October to July. Prior to exposure to bulls, cows would have

breeding tags[4] attached to them to document if bulls have mounted them to breed. Following first exposure of cows to bulls in 2012, five calving seasons would be observed (2013-2017). In February of each year, cows would be pregnancy-tested and fitted with vaginal transmitters to alert investigators to abortion or calving events.

During the abortion/calving seasons (from February until August of each year), daily observation for abortions, labor, and calving events would be conducted by study investigators. Within five days of abortion or calving, the cow would be immobilized and blood, milk, and vaginal swabs would be collected for testing. If possible, the calf would also be captured and eye swabs and blood would be collected for testing.

Following an abortion, the fetus would be left at the abortion site for 24 hours to monitor exposure to other bison. The fetus would then be collected, tested, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, Montana.

Blood testing of cows, bulls, and calves would be conducted three times a year: in February, calving time, and in the fall. Blood would be analyzed at the MVDL and/ or the National Veterinary Service Laboratories in Ames, Iowa throughout the study to determine *B. abortus* infection status of each animal.

Handling and physical restraint of bison for tagging or blood collection would take place in alleyways leading to standard bison manual squeeze chutes. Injection of the study animals with GonaCon™ would be done by study personnel experienced in administering intramuscular vaccines. Blood samples from study animals would be collected using established techniques for collection of blood from bison and would be performed by study personnel experienced with these techniques. An attending veterinarian would be available to address concerns about animal care and use for the study.

When the study is completed, all seropositive animals would be humanely euthanized following American Veterinary Medical Association-approved guidelines, and specimens would be collected from each animal for laboratory analysis. In addition, eggs and semen would be collected from these animals and frozen for genetic conservation. Per the conditions of the approval from EPA to use GonaCon™ in bison in this confined experimental use study, animals treated with GonaCon™ cannot be consumed by humans. These animals would be disposed of by incineration or landfill burial. Seropositive animals from the study that have not received GonaCon™ injections would be distributed to Montana food

[4] Breeding tags are devices that are temporarily adhered to the base of the cow's tail that indicate by a color change that the cow has been mounted.

banks as is routinely done with other YNP seropositive bison. Further discussion on the safety of consuming bison infected with *B. abortus* is discussed in the human health and safety section of this document. All animals that test negative for brucellosis for the duration of the study and satisfy existing bison quarantine release requirements outlined in the APHIS Uniform Methods and Rules (USDA APHIS, 2003) would be used for bison conservation purposes.

C. Other Alternatives Considered but Dismissed from Further Consideration

Because the most common route of transmission of *B. abortus* is contact with infected birthing fluids, aborted fetuses, and placental tissues, different methods of impacting the fertility of bison through the use of immunocontraceptive vaccines were considered as alternatives to the proposed action. If pregnancy could be prevented in *B. abortus*-infected female bison, transmission of *B. abortus* by this route could be eliminated or decreased.

APHIS considered the use of Porcine zona pellucida (PZP), another type of immunocontraceptive vaccine that has been used in animal species such as dogs, coyotes, burros, wild horses, and deer (USDA APHIS, 2010b). PZP has also been demonstrated to effectively induce temporary infertility in captive bison (Frank et al., 2005). However, research has shown that the use of PZP can increase the period of time in which the treated animals exhibit breeding season behavior.

The PZP vaccine results in temporary infertility while still allowing female animals to have multiple estrous cycles in which they engage in prebreeding behavior and breed. This behavior can cause animals to use energy at times of the year, such as late fall and early winter, when they would otherwise be conserving energy. Miller et al. (2004) concluded that "…Prolonging the breeding season of bison in the GYA may be deleterious to the winter survival of dominant bulls and PZP vaccinated cows because of increased sexual activity during fall and early winter." Therefore, this alternative was dismissed from further consideration because investigating the use of a PZP vaccine would not be useful in brucellosis management strategies in bison since it is associated with increased mating and reproductive activity (Killian et al., 2007).

APHIS also considered the alternative of physical sterilization as a means of decreasing the transmission of *B. abortus* within bison populations and between bison and cattle in the GYA. Physical sterilization such as spaying[5] or castration[6] has been recognized as a disease management

[5]Surgical removal of the ovaries from female bison.

strategy that could be used to reduce the potential transmission of brucellosis in infected wildlife populations. However, this type of sterilization is permanent. APHIS would not subject the bison in the study to physical sterilization. For this reason, this alternative was dismissed from further consideration.

III. Potential Environmental Impacts

The NEPA implementing regulations provide criteria that Federal agencies should evaluate, if applicable, in environmental documents for proposed actions. This section of the EA addresses the applicable criteria related to potential impacts from the no action alternative and from the proposed action. NEPA criteria that are applicable for consideration in this section of the document include animal impacts, human health and safety, and the physical environment.

A. No Action

Without the proposed action, efforts to gather scientific information to better understand the potential application of immunocontraceptive vaccines such as GonaCon™ as a nonlethal strategy for reducing the transmission of *B. abortus* and decreasing the prevalence of brucellosis in the wild bison population in YNP would be lost. Without the proposed action to assist in developing nonlethal strategies to effectively control and eliminate brucellosis, the disease may continue to spread within the wild, free-ranging bison population in the GYA.

B. Proposed Action

1. Impact of Proposed Action on Animals

a. Bison

The proposed study would not increase the risk of brucellosis being transmitted within the bison population. Therefore, this section focuses on the potential effects of the admistration of GonaCon™ in bison.

In this proposed study, the desired effect of administering GonaCon™ is the temporary suspension of reproductive activity in the vaccinated female bison. Miller et al. (2004) report that "The gonadotropin-releasing hormone (GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns as the GnRH antibody titer drops below a protective level." If the effect of this immunocontraceptive vaccine successfully places the vaccinated

[6] Surgical removal of the testes of male bison.

bison cows in a temporary nonreproductive state, the transmission of brucellosis by the female bison via shedding of *B. abortus* during calving or abortion may be eliminated.

A small study conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho in 2002-2003 demonstrated "that a single injection of GnRH vaccine is effective in preventing conception in female bison for at least 1 yr" (Miller et al., 2004). In that study, three of the six GnRH-treated bison cows and five of the untreated bison cows were in the last month of pregnancy at the time of vaccination. They delivered normal calves in the first year, suggesting that the GnRH vaccine did not interfere with the pregnancy and could be administered safely during the last third of the pregnancy. Additionally, none of the six treated bison became pregnant during the first breeding season (Miller et al., 2004).

Undesired health effects have been minimal in the species of wildlife in which GonaCon™ has been used. Injection site reactions caused by the "water-in-oil (W/O) emulsion needed in the GonaCon™ formulation for development of a long-term immune response" have been observed (Miller et al., 2008b). These reactions were most commonly manifested as inflammation or swelling at the injection site, or the presence of granulomas (thickened tissue filled with fluid). This observation is not uncommon in other livestock vaccines (USDA APHIS, 2010b).

As part of the GonaCon™ EPA registration process for use in deer, the health effects to the vaccinated deer were evaluated. Vaccinated animals showed no external evidence of inflammation at known injection sites; however, when muscle tissue at the injections site was sectioned, the injection sites appeared to be comprised of whiteish scar tissue, some containing vesicles of sterile fluid. All blood chemistry analyses were similar between treated and untreated deer. (Killian et al., 2006). Other types of injected products that alter animal hormones are currently used in livestock in the United States (USDA APHIS, 2010b).

Ensuring humane handling and treatment of all bison during the proposed study activities would be a priority. Application of animal identification tags, administration of GonaCon™ vaccine, and evaluation of pregnancy status, calving, or abortion `activities would be conducted at appropriate times during the proposed study. These activities would be overseen by the study's attending veterinarian and would not be expected to cause more than momentary or slight pain or discomfort. All temporary restraining and handling or temporary immobilization and handling activities would be conducted as quickly and efficiently as possible and in a manner that would prevent undue stress, trauma, injury, or any unnecessary discomfort to the animal. If temporary immobilization is necessary, bison cows would be immobilized in locations within the

facilities that are safe for the animals and the proposed study personnel. Veterinary oversight for animal care and handling, restraint, and sample collection would be provided during the proposed study activities. Wildlife biologists trained and experienced in the handling of bison would also be participating in the proposed study activities.

If necessary, study personnel would use the Federal Drug Administration (FDA)-approved anaesthetic and pain-killing (analgesic) drug combinations to immobilize the animals in order to prevent any potential negative impacts to the bison during the collection of study samples. The immobilization drugs would be used according to standard animal administration techniques, and it is expected that the bison would be immobilized for no more than 20 minutes. Vital signs of the immobilized bison would be monitored by qualified study staff throughout the sampling procedures and the initial recovery phase. To further ensure humane handling of the bison, every precaution would be taken by study staff to prevent immobilization- or handling-related trauma, injury, or death to the bison. The standard chemical immobilization protocol that would be used in this proposed study is widely used in bison and other wild ungulates without long-term effects (Kreeger et al., 2002).

In the GonaCon™ EPA registration process for use in deer, concerns were initially raised by some States that GonaCon™ would eliminate the need to use hunting as a tool to control deer overpopulation. Contraception alone would not reduce overabundant deer populations to healthy levels (USDA APHIS, 2010b). In deer, GonaCon™ is meant to be used in combination with other wildlife management tools to control populations. Assuming the use of GonaCon™ is eventually registered by EPA for bison, it is equally implausible to conclude that use of the contraceptive vaccine in bison would result in any significant population decreases in bison in the absence of other management tools (USDA APHIS, 2010b).

b. Non-Target Species

The proposed study would not increase the risk of brucellosis being transmitted to non-target species. Therefore, this section focuses on the risk of non-target species being exposed to GonaCon™.

In the proposed study, it is unlikely that non-target species would be exposed to GonaCon™. The proposed study protocol includes both risk mitigation measures that prevent direct exposure of non-target species to GonaCon™ and measures that limit the potential for secondary exposure of non-target species to GonaCon™.

To prevent direct exposure to non-target species, GonaCon™ would be administered directly to the study bison by hand-injection with a syringe.

By using this direct-injection method, there would be no potential for accidental injection of non-target species with GonaCon™.

To prevent the risk of secondary exposure, the study plan includes measures to restrict access to treated animals by predators or other non-target species. To prevent access by larger wild animals, the bison in the proposed study would be maintained in double-fenced pastures, not on open range, thereby physically limiting potential contact between treated bison and wild animals such as elk, bears, and coyotes.

Abortions or calving events by GonaCon™-treated bison should be very minimal since the expected effect of treatment with GonaCon™ is to prevent pregnancy. The proposed study protocol includes actions to detect abortion or calving events, and the fencing would also physically limit some wild animals from accessing infected bison tissues from the GonaCon™-treated bison. The study protocol also includes standard operating procedures for proper removal and disposal of *B. abortus*-infected animal tissues from GonaCon™-treated bison from the study area to further limit potential exposure.

As discussed above, some larger animal species can be physically prevented from accessing the study area. However, some species such as birds of prey, smaller rodents, or insects cannot be prevented from accessing the study area. In the event that a non-target species were to consume GonaCon™-treated infected bison carcasses or GonaCon™-treated *B. abortus*-infected animal tissues, there would be no anticipated adverse effects from the GonaCon™ vaccine. Because GonaCon™ is made of proteins, it is broken down into smaller amino acids through digestion when it is consumed and has no contraceptive effect on non-target species (Fagerstone et al., 2008; Fagestone et al., 2010).

As part of the registration process for the use of GonaCon™ in deer, EPA concluded that there is no known danger associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007). Similar injectable hormone-altering products are used routinely in livestock applications (USDA APHIS, 2010b).

2. Human Health and Safety

a. General Public

The proposed study discussed in this EA would be conducted on double-fenced, private facilities where access by the general public to bison and potentially infected animal tissues such as aborted fetuses or reproductive materials would be prohibited. The protocol for the study contains standard operating procedures for handling and safely disposing of any potentially brucellosis-infected materials generated from the animals in the study. The general public would have no risk of being exposed to either

GonaCon™ -treated or untreated animals from the study or any potentially infected materials generated from the study.

There is no danger of transmission of the infection to humans from consuming cooked meat from *B. abortus*-infected bison. The *B. abortus* bacteria that causes brucellosis is typically not found in muscle tissue, and normal cooking temperatures kill any existing bacteria (USDA APHIS, 2011). Additionally, EPA and FDA concluded that there are no known human food safety concerns associated with eating deer that have been vaccinated with GonaCon™ (USEPA, 2007 and FDA, 2005).

b. Worker Safety

Personnel who would be involved in the proposed study are qualified and have the expertise and experience needed to carry out the study activities. These activities include wildlife chemical immobilization, proficiency in administration of animal vaccines, veterinary care, animal restraint, tagging and marking animals, sample collection, and field evaluation of reproductive behaviors and activities.

Standard operating procedures would be in place to protect personnel involved in carrying out the proposed study activities. The standard operating procedures would include measures for safe and humane handling of bison to prevent injury to study personnel and to bison; safe handling and administration of GonaCon™; safe and humane collection of study samples for analysis; and safe handling procedures for study samples, including the safe handling and proper disposition of potentially infected animal tissues. In addition to the standard operating procedures and safety measures, at least one cell phone would be available at all times to facilitate contact in emergencies, and first aid kits would be available at all times in the event of injury to study personnel.

The GonaCon™ immunocontraceptive vaccine would be provided for the study in pre-mixed syringes and stored in locked containers except when actively being used to inject study animals. Personnel handling the vaccine would take appropriate precautions to prevent accidental self-injection. Pregnant women would not be involved in the handling or injecting of GonaCon™ at any time during the proposed study to avoid any potential risks associated with accidental exposure to the immunocontraceptive vaccine. Immobilization drugs and associated reversal drugs would be available for use if needed in the study. These drugs would be properly stored in locked containers to prevent improper access.

3. Physical Environment

As previously mentioned, proposed study activities would occur in several pastures at the Brogan Bison Facility, just north of Corwin Springs

(60 acres), and the Slip ‘n Slide pasture (25 acres) and/or Rigler pasture (32 acres), located north of Gardiner, Montana.

The Brogan Bison Facility is used by APHIS VS for bison research. Forage at the pastures includes a mix of cultivated and native grasses. The upper pasture is on a steep slope along the west side of the property with several grass benchlands[7] and steep, rocky drainages. The vegetation is composed of thinly forested slopes, interspersed with native bunchgrass rangelands (MFWP, 2005). Bassett Creek runs through the property and flows into the Yellowstone River.

The Slip ‘n Slide and Rigler pastures are located in close proximity to each other, just south of Yankee Jim Canyon. The pastures are double-fenced. The landscape is gently sloping and consists mostly of native grassland, except for the mixed alfalfa- and grass-cultivated hay meadows. Slip ‘n Slide Creek runs through the Slip ‘n Slide property and flows into the Yellowstone River. There are no brooks or creeks running through the Rigler pastures. The pastures are primarily surrounded by Gallatin National Forest and State of Montana land. Montana Fish, Wildlife and Parks historically leases the pastures on the ranch for bison to graze on (MFWP, 2011).

The potential environmental impacts of the proposed study on the physical environment would primarily be due to bison grazing in confined areas. The main issues of concern regarding confined grazing are effects on soil, vegetation, and water quality. These aspects are discussed below.

a. Soil and Vegetation

Livestock grazing in confined pastures can negatively affect soil quality by compacting the soil or causing soil erosion due to loss of vegetation cover. With a loss of vegetation, invasive species also threaten pastures. Most studies and discussions on the impacts of grazing focus on cattle because 70% of the western United States is grazed by livestock, which is primarily composed of cattle (Fleischner, 1994). Cattle are similar to bison in that they are large generalists and ungulate herbivores that can disturb terrestrial communities; however, differences in the two animals, such as forage selection and social organization (Hartnett et al., 1997; Steuter and Hidinger, 1999), may influence their impacts on soil and vegetation.

Bison have a stronger preference for perennial grasses than cattle. Cattle consume a higher percentage of forbs[8] in their diet than bison, and they

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

[8] Herbaceous flowering plants other than grass.

use wooded areas and riparian zones more intensively than bison (Steuter and Hidinger, 1999). Due to the lower diversity of plants consumed by bison and the bison's preference for herbaceous vegetation, there may be a reduction in the abundance of dominant grasses, an increase in the survival of subordinate species, and an increase in species diversity, when compared to land grazed by cattle (Hartnett et al., 1997). Additionally, physical disturbances that bison exhibit during non-grazing activities, such as wallowing[9] may assist in ecodiversity (Hartnett et al., 1997).

The proposed action would not alter historic land use (for information regarding historic or cultural sites, see section below in the section on other environmental review requirements) at the pastures; therefore, overall effects to soil and vegetation would not be increased. Approximately 100 bison would be placed on 120 irrigated acres of land, averaging about one acre of land per bison. This density is expected to have only minimal impacts on the land. In addition, landowners at each ranch or facility implement management practices to minimize effects to soil and vegetation. Pasture rotation is practiced at or between facilities as necessary, so that each pasture is periodically rested and the land is not overused. Lastly, the bison at all facilities would be supplemented with hay, further limiting pasture grazing.

b. Water

GonaCon™ is a protein that is broken down within the treated bison; its metabolites would not be anticipated to be any greater than what would naturally occur. Therefore, this section focuses on other potential environmental impacts of bison grazing near water.

Potential environmental impacts from bison grazing in pastures could include a degradation of nearby water quality by manure, urine, and sediment being deposited into local waters. While bison that have access to a water body may directly deposit manure and urine into the water, wastes excreted onto land may also be transported to water bodies via leaching and surface runoff.

Grazing management practices can lessen the environmental impacts of streamside pastures. While many studies describe the impact of cattle grazing on water bodies, few studies have concentrated on the effects of native ungulates on stream health. Russell et al. (2009) states that the proximity of cattle to the stream, the amount of time they spend by or in the stream, and the intensity and length of cattle grazing can all influence

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

the water quality of nearby streams. One can assume the same behaviors in bison would also impact water quality.
Bison spend less time in streams or riparian habitats than cattle (Fleischner, 1994). Fleischner describes a study conducted in Utah regarding the feeding ecology of cattle and bison. The study noted that "cattle distribution was limited to gentle slopes near water, regardless of forage, while bison roamed widely, seemingly unaffected by slope or proximity to water." As previously mentioned, cattle forage on a higher percentage of forbs and woody vegetation and maintain a larger breadth of diet niche than bison. Fritz et al. (1999) takes this one step further and states that a higher percentage of forbs and woody vegetation occurs in the riparian zone, so cattle are more likely to impact stream riparian zones than bison.

Fritz et al. (1999) studied the distribution and diversity of macroinvertebrates (e.g., insects, worms, snails and crayfish) in relation to bison crossings in prairie streams. The study suggests that impacts of bison on communities at the bottom of the streams was spatially limited, and that the bison may have less impact on stream communities than other studies of the impact of cattle. While comparison to cattle provides a noteworthy point of reference, it is important to point out that it is difficult to compare environmental responses with cattle versus bison due to confounding effects of site, weather, and management.

The pastures that would be utilized in the proposed study have historically been used for bison research or as livestock pastures, so deposits of manure, urine, and sediment due to the proposed study are not expected to increase the historic amount of contaminants entering the Yellowstone River. While the Brogan Bison Facility does have a creek running through it, bison do not have access to the creek. Only bison at the Slip 'n Slide ranch would have direct, but limited, access to a creek. The access site to this creek was historically used for livestock and is at a point on the creek where the bank is shallow and covered with rocks. A shallow crossing means that bison would not have to climb up and down the bank, which would eventually cause the banks to erode. In addition, water would be provided to the bison, limiting the time that bison would visit the creek. Lastly, because only a portion of the total number of bison tested would be present on this pasture and those bison would spend limited time in streamside environments, the impact to water bodies is expected to be minimal.

IV. Other Environmental Review Requirements

A. Endangered or Threatened Species

Section 7 of the Endangered Species Act (ESA) and its implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of endangered or threatened species or result in the destruction or adverse modification of critical habitat. Proposed study activities would occur in pastures in southern Park County in Montana.

There are two federally listed mammals in Park County: the Canada lynx (*Lynx canadensis*) and the grizzly bear (*Ursos arctos horribilis*). Critical habitat has been designated for the Canada lynx in Park County.

Canada lynx: Areas designated as critical habitat for the Canada lynx include boreal forest landscapes that provide one or more of the following primary constituent elements for the lynx: snowshoe hares for prey; abundant, large, woody debris piles that are used as dens; and winter snow conditions that are generally deep and fluffy for extended periods of time (USDOI FWS, 2009).

Grizzly bear: In Montana, grizzly bears primarily use meadows, seeps, riparian zones, mixed shrub fields, closed timber, open timber, sidehill parks, snow chutes, and alpine slabrock habitats. Habitat use is highly variable between areas, seasons, local populations, and individuals. Grizzly recovery zones (areas identified where grizzly bear distribution is primarily within), including the Yellowstone area in northwest Wyoming, eastern Idaho, and southwest Montana (9,200 square miles), are estimated at more than 580 bears (FWS, 2011).

At all three locations, the pastures are double-fenced with an 8-foot woven wire fence and an electric high tensile fence to contain the study bison. These fences would also prevent Canada lynx and grizzly bears from entering the pastures. If Canada lynx or grizzly bears were to enter the pastures and consume GonaCon™-treated bison, there would be no effect on these species. The vaccine is made of proteins, and when consumed, is broken down into amino acids in the gastrointestinal tract, thereby having no contraceptive effect (Fagerstone et al., 2008; Fagerstone et al., 2010).

Federally-listed species and other non-target wildlife would not be directly exposed to GonaCon™ because the vaccine would be injected directly into the test bison and not administered orally in bait form. No wildlife habitat would be altered or disrupted by proposed study activities. No

helicopters would be used as part of this proposed study; therefore, no disturbance to wildlife in the surrounding area is expected. Although the study pastures occur within the designated critical habitat of the Canada lynx, the proposed study would have no effect on the primary constituent elements of that habitat and would not adversely modify it. Therefore, APHIS has determined that the proposed action would have no effect on the grizzly bear or Canada lynx.

B. Bald and Golden Eagle Protection Act

The Bald and Golden Eagle Protection Act (16 U.S.C. 668-668c) prohibits anyone, without a permit issued by the Secretary of the Interior, from "taking" bald eagles, including their parts, nests, or eggs. The Act provides criminal penalties for persons who "take, possess, sell, purchase, barter, offer to sell, purchase or barter, transport, export or import, at any time or any manner, any bald eagle ... [or any golden eagle], alive or dead, or any part, nest, or egg thereof." The Act defines "take" as "pursue, shoot, shoot at, poison, wound, kill, capture, trap, collect, molest or disturb."

There are no known bald eagle nests around the facilities; nesting areas are further down river (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.). However, golden eagle nests could be in the vicinity of the facilities, although specific nests are not known. Therefore, the proposed study is not expected to have any impact on nesting bald or golden eagles. In addition, activities occurring during the proposed study would not differ significantly from activities normally occurring at these pastures. “Eagles are unlikely to be disturbed by routine use of roads, homes, and other facilities where such use pre-dates the eagles’ successful nesting activity in a given area. Therefore, in most cases ongoing existing uses may proceed with the same intensity with little risk of disturbing bald eagles” (FWS, 2007). If study personnel discover the presence of any bald or golden eagle nests in the area, this information would be reported to the Wildlife Program Manager at Gallatin National Forest.

Golden eagles have been observed flying over the Brogan Bison Facility (Jeremy Zimmer, USDA, Forest Service, Gardiner, MT, pers. comm.) and bald eagles could be flying in the area as well. The activities that would occur during the proposed study would not differ significantly from activities that normally occur in these pastures. Therefore, no disturbance of eagles would occur as a result of the proposed study; eagles in the area would be accustomed to these activities.

Although program personnel would remove daily any aborted calves or treated or non-treated bison that could die during the study, bald and golden eagles in the area could potentially consume these items. However, “[i]mmunocontraception vaccines provide few risks for

consumptive use of dosed wildlife; the antibodies that prevent reproduction are only one of millions of other antibodies present in animals, all of which are harmless to the organism that digests them, like any other proteinaceous food consisting of amino acids" (Fagerstone et al., 2010). Therefore, no eagles would be harmed if consumption of these items occurred.

C. Historic and Cultural Resources

In accordance with Section 106 of the National Historic Preservation Act of 1966 and its implementing regulations[10], APHIS prepared a summary of the proposed project and submitted it to the Montana State Historic Preservation Office (SHPO) for consideration of potential impacts to historic resources. On November 28, 2011, APHIS received a letter of concurrence from the Montana SHPO agreeing that there were no findings of potential impacts to historic resources for the proposed study.

D. Tribal Consultation and Coordination

In accordance with Executive Order 13175: Consultation and Coordination with Indian Tribal Governments[11], APHIS has prepared a summary of the proposed project and provided it to 26 tribes who may have interests in YNP. In addition to the 26 identified tribes, APHIS also provided a summary of the project to all tribes located near YNP and in States adjacent to Montana who might potentially have interest in the project.

On December 19, 2011, APHIS held a conference by telephone with tribes to provide an opportunity to discuss the proposed project in more detail and discuss potential concerns that the tribes may have. Tribes that participated in the call showed an interest in the details of the project, and several requested additional information on the history of the GonaCon™ immunocontraceptive vaccine. APHIS agreed to provide background information to tribes. No tribes voiced any major concerns about the project.

APHIS will continue to conduct outreach to interested tribes and keep them updated on the activities associated with the project.

[10] National Historic Preservation Act of 1966 (16 U.S.C. 470f) and implementing regulations (36 CFR §800).

[11] Executive Order 13175: Consultation and Coordination with Indian Tribal Governments (65 FR 67249, November 9, 2000).

V. Cumulative Impacts

This EA examines the activities associated with a proposed study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the YNP bison population by effecting temporary infertility in bison cows and thereby preventing transmission of *B. abortus*. Activities associated with the proposed study are not expected to result in adverse cumulative effects.

In order to conduct the proposed study, approximately 96 female and 8 male bison that naturally exit YNP over the period of as many as three years would be housed at pasture locations in the Gardiner, Montana area. Some of the female animals in the study would be injected with GonaCon™, which would reduce the likelihood of pregnancy and delivery of offspring in the treated animals. Untreated females may give birth to offspring, which would increase the total number of animals associated with the study.

In August 2011, the National Park Service conducted an annual bison population estimate (NPS, 2011). According to the 2011 survey, the total bison population in YNP was estimated to be approximately 3,700 bison. This total was approximately 200 lower than the survey from the previous summer, but the decrease was "within the natural range of expectation for wild bison."

Assuming the proposed study would result in approximately 104 bison being removed from the larger bison population of YNP, the effect of removing this number of bison over multiple years is well within the natural range of expectation for bison. This decrease in the numbers of bison in YNP is not anticipated to cause any cumulative negative effects to the overall bison population.

One of the goals of the IBMP is to manage temporal and spatial separation of bison and cattle to mitigate potential transmission of brucellosis. Currently, this is accomplished through hazing, capture, test and slaughter of seropositive animals, and vaccination of seronegative animals and a limited hunt in Montana. The proposed study may provide important information that would allow for re-evaluation and re-consideration of some of the current IBMP activities. This may result in impacts to future decision-making regarding protocols for bison habitat management, bison vaccination strategies, and bison hunt activities. IBMP activities that could be impacted include strategies to maintain appropriate bison population and distribution, should bison habitat be expanded.

VI. Agencies or Persons Contacted

U.S. Forest Service, Gallatin National Forest

Montana Fish, Wildlife and Parks

Montana State Historic Preservation Office, Montana Historical Society

USDA, Animal and Plant Health Inspection Service, Veterinary Services

USDA, Animal and Plant Health Inspection Service, Policy and Program Development, Environmental and Risk Analysis Services

VII. References

Aune, K., J.C. Rhyan, R. Russell, T.J. Roffe, and B. Corso. 2011. Environmental persistence of *Brucella abortus* in the Greater Yellowstone Area. The Journal of Wildlife Management 9999:1-9.

Cheville, N.F., D.R. McCullough, and L.R. Paulson. 1998. Brucellosis in the Greater Yellowstone Area. National Research Council. National Academy Press. Washington, DC 186pp.

Clarke, R., Jourdonnais, C., Mundinger, J., Stoeffler, L., and R. Wallen. 2005. A Status Review of Adaptive Management Elements, 2000 to 2005. Interagency Bison Management Plan. National Park Service; United States Department of Agriculture, Animal and Plant Health Inspection Service; United States Department of Agriculture, Forest Service; Montana Department of Livestock; and, Montana Fish, Wildlife and Parks.

DeYoung, J., and R. Leep. 2011. Grazing Streamside Pastures. Michigan State University. http://fis.msue.msu.edu/extension_documents/Grazing_Streamside_Pastures.htm

Fagerstone, K.A., L.A. Miller, J.D. Eisemann, J.R. O'Hare, and J.P. Gionfriddo. 2008. Registration of wildlife contraceptives in the United States of America, with OvoControl and GonaCon™immunocontraceptive vaccines as examples. Wildlife Research. 35:586-592.

Fagerstone, K.A., L.A. Miller, G. Killian, and C.A. Yoder. 2010. Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human-wildlife conflicts in North America. Integrative Zoology. 1:15-30.

Fleischner, T.L. 1994. Ecological costs of livestock grazing in western North America. Conservation Biology. 3(8):629-644.

Food and Drug Administration (FDA). 2005. Human food safety evaluation of the proposed formulation of GonaCon™ Immunocontraceptive Vaccine for White-Tailed Deer. Letter from FDA's Department of Health & Human Services to USDA APHIS' Policy and Program Development. November 30, 2005.

Frank, K.M., R.O. Lyda, and J.F. Kirkpatrick. 2005. Immunocontraception of captive exotic species. IV. Species differences in response to the Porcine Zona Pellucida Vaccine, timing of booster inoculations, and procedural failures. Zoo Biology. Volume 24: 349-358.

Fritz, K.M., W.K. Dodds, and J. Pontius. 1999. The effects of bison crossings on the macroinvertebrate community in a tallgrass prairie stream. Am. Midl. Nat. 141: 253-265.

FWS – see U.S. Fish and Wildlife Service

Gionfriddo, J.P., J.D. Eisemann, K.J. Sullivan, R.S. Healey, L.A. Miller, K.A. Fagerstone, R.M. Engeman, and C.A. Yoder. 2009. Field test of a single-injection gonadotrophin-releasing hormone immunocontraceptive vaccine in female white-tailed deer. Wildlife Research 36:177-184.

Gionfriddo, J.P., A.J. DeNicola, L.A. Miller, and K A. Fagerstone. 2011a. Efficacy of GnRH immunocontraception of wild white-tailed deer in New Jersey. Wildlife Society Bulletin 35:142-148.

Gionfriddo, J.P., A. J. DeNicola, L. A. Miller, and K. A. Fagerstone. 2011 (b). Health effects of GnRH immunocontraception of wild white-tailed deer in New Jersey. Wildlife Society Bulletin 35:149-160.

Hartnett, D.C., A.A. Steuter, and K.R. Hickman. 1997. Comparative ecology of native and introduced ungulates. pp. 72-101. *In* F. Knopf and F. Samson (eds.) Ecology and Conservation of Great Plains Vertebrates, Springer-Verlag, New York.

Interagency Bison Management Plan Operating Procedures (IBMPOP). 2009. http://ibmp.info/Library/Operating%20Procedures/2009-10%20Operating%20Procedures.pdf *last accessed* January 5, 2012.

Killian G., J. Eisemann, D. Wagner, J. Werner, D. Shaw, R. Engeman, and L. Miller. 2006. Safety and toxicity evaluation of GonaCon™) immunocontraceptive vaccine in white-tailed deer. Proceedings of the Vertebrate Pest Conference 22:82-87.

http://www.aphis.usda.gov/wildlife_damage/nwrc/publications/06pubs/eisemann062.pdf *last accessed* January 13, 2012.

Killian G., K. Fagerstone, T. Kreeger, L. Miller, and J. Rhyan. 2007. Management strategies for addressing wildlife disease transmission: the case for fertility control. Proceedings of the 12th Wildlife Damage Management Conference (D.L. Nolte, W.M. Arjo, D.H. Stalman, eds). 2007. Wildlife Damage Management, Internet Center for USDA National Wildlife Research Center – Staff Publications. University of Nebraska – Lincoln.

Killian, G. D.Thain, N.K. Diehl, J. Rhyan and L. Miller. 2008. Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and interuterine devices. Wildlife Research 35:531-539.

Kreeger, T.J., J.M. Arnemo, and J.P. Raath. 2002. Handbook of Wildlife Chemical Immobilization. International Edition. Wildlife Pharmaceuticals, Inc., Fort Collins, CO 412pp.

Meyer, M.E., and M. Meagher. 1995. Brucellosis in free-ranging bison (*Bison bison*) in Yellowstone, Grand Teton, and Wood Buffalo National 17 Parks: A review. (letter to the editor) Journal of Wildlife Diseases. 31:579-598.

MDoL – See Montana Department of Livestock

MFWP – See Montana Fish, Wildlife & Parks

Miller, L.A., J.C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. Journal of Wildlife Diseases. 40(4):725-730.

Miller, L.A., J. Gionfriddo, K. Fagerstone, J. Rhyan, and G. Killian. 2008a. The single-shot GnRH immunocontraceptive vaccine (GonaCon™) in white-tailed deer: comparison of several GnRH preparations. American Journal of Reproductive Immunology. 60:214-223.

Miller, L., K. Fagerstone, J. Kemp, G. Killian, and J. Rhyan. 2008b. Proceedings of the 23rd Vertebrate Pest Conference (R.M. Timm and M.B. Madon, eds.) University of California, Davis. pp.244-249.

Montana Department of Livestock (MDoL) and Montana Fish, Wildlife & Parks (MFWP). 2000. Interagency Bison Management Plant for The State of Montana and Yellowstone National Park: Final Environmental Impact Statement. November 15, 2000.

Montana Fish, Wildlife & Parks (MFWP). 2005. Draft Environmental Assessment for Bison Quarantine Feasibility Study Phase II/III. December 15, 2005. http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/bison_quarantine_ea-draft.pdf *last accessed* November 4, 2011.

Montana Fish, Wildlife & Parks (MFWP). 2011. Draft Environmental Assessment for Interim Translocation of Bison. September, 2011. http://fwpiis.mt.gov/content/getItem.aspx?id=52297 *last accessed* November 4, 2011.

National Park Service (NPS). 2011. Yellowstone National Park News Release: Yellowstone's Summer 2011 Bison Population Estimate Released. August 16, 2011. Retrieved 12/01/2011 from http://www.nps.gov/yell/parknews/11086.htm.

Russell, J., D. Bear, K. Schwarte, and M. Hann. 2009. Grazing Management of Beef Cows to Limit Non-Point Source Pollution of Streams in Midwestern Pastures. Iowa State University.

Steuter, A. and L. Hidinger, 1999. Comparative ecology of bison and cattle on mixed-grass prairie. Great Plains Studies, Center for Great Plains Research: A Journal of Natural and Social Sciences. 9(2):329-342.

U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). 2003. Brucellosis Eradication: Uniform Methods and Rules, Effective October 1, 2003, APHIS 91–45–013. 121pp. http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/umr_bovine_bruc.pdf *last accessed* October 21, 2011.

U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). 2010a. Veterinary Services National Brucellosis Surveillance Strategy, December 2010, 8pp. Retrieved 10/4/2011 from http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/natl_bruc_surv_strategy.pdf.

U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). 2010b. Factsheet: Questions and Answers: GonaCon™–Birth Control for Deer, 3pp. http://www.aphis.usda.gov/wildlife_damage/nwrc/publications/factsheets/FS_FAQ_GonaCon™ May%202010.pdf *last accessed* September 20, 2011.

U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). 2011. Facts About Brucellosis, 7pp.

http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/bruc_facts.pdf *last accessed* December 13, 2011.

U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). 2012. Brucellosis and Yellowstone Bison. Retrieved on 1/4/2012 from http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved

U.S. Department of the Interior, Fish and Wildlife Service. 2009. Endangered and Threatened Wildlife and Plants; Revised Designation of Critical Habitat for the Contiguous United States Distinct Population Segment of the Canada Lynx. Federal Register, Vol. 74, p. 8616–8702, February 25, 2009.

USDOI FWS—see U.S. Department of the Interior, Fish and Wildlife Service

USEPA – See U.S. Environmental Protection Agency

U.S. Environmental Protection Agency. 2007. Experimental use permit for GonaCon™) immunocontracpetive vaccine for deer. Memorandum from Kit Farwell, Reregistration Branch 1 to Joanne Edwards, Registration Division. July 3, 2007.

U.S. Fish and Wildlife Service. 2007. National Bald Eagle Management Guidelines. 23 pp. Available http://www.fws.gov/pacific/eagle/NationalBaldEagleManagementGuidelines.pdf *last accessed* September 30, 2011.

U.S. Fish and Wildlife Service. 2011. Grizzly bear recovery home page. Mountain-Prairie Region, Endangered Species Program. Available http://www.fws.gov/mountain-prairie/species/mammals/grizzly/ *last accessed* November 14, 2011.

LEGAL NOTICE

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

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is making available to the public an environmental assessment for a proposed study to evaluate whether GonaCon™, an immunocontraceptive vaccine, would be effective as a non-lethal method of decreasing the prevalence of brucellosis in the Yellowstone National Park bison population. This proposed action is planned for locations on private ranch land near Gardiner, Montana. The environmental assessment, "Evaluation of GonaCon™, an Immunocontraceptive Vaccine, as a Means of Decreasing Transmission of *Brucella abortus* in Bison in the Greater Yellowstone Area," is available online at http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/ and http://www.ibmp.info. Paper copies may be obtained by contacting USDA APHIS, Veterinary Services Area Office, 208 North Montana Avenue, Suite 101, Helena, MT 59601 or (406) 449-2220.

Comments may be submitted via email to EAComments2012@aphis.usda.gov or by mail to the VS Area Office listed above. Comments must be received by February 25, 2012. For more information about the study, please contact the VS Area Office at (406) 449-2220.

LEGAL NOTICE

Hypotheses:

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

1.1 United States Department of Agriculture

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

PROTOCOL COVER PAGE

Study Title:	**Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison**
NWRC Study Director:	Jack Rhyan
Approved NWRC Project:	Development of injectable and oral contraceptive technologies and their assessment for wildlife population and disease management

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☒ Cover Page ☒ Part 1 (Signature Page) ☒ Part 2 (Regulatory Considerations) X☒ Part 4 (full NWRC Study Protocol) ☐Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: 2/17/12

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☒ Research Scientist

☐ Project Leader

☑ Visiting Scientist: NWRC Representative/Contact: LOWELL MILLER

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date 2/17/12

Review and Processing:
QAU: _____ Date 2/21/12

Concur:
NWRC Assistant Director _____ Date 2/22/12

Approved:
NWRC Director _____ Date 2/22/12

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix.**
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____National Park Service_____ _YELL-2011-SCI-5892_____ May 10, 2011____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix.** Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _June 2, 2011_____
☒	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☒	☐	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☐	☒	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement**. Material Transfer agreements will be developed prior to material transfer
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)?

		If yes, attach **Analytical Chemistry Appendix.**

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Jenny Powers	NPS	Collaborator
Rick Wallen	NPS	Collaborator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Serologic testing; fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Manufacture of vaccine, Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: April 15, 2012
Proposed Experimental Termination Date: October1, 2017
Proposed Study Completion/Archive Date: October 1, 2019

5. Background and Justification

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

through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison is unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg (Miller et al., 2004). Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus.*

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and other species (Miller et al., 2000; Miller et al., 2004; Miller et al., 2008; Killian et al., 2009; Yoder and Miller, 2010). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed and Scopus on 12/29/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison, immunocontraception and bison, GnRH and brucellosis, GonaCon and brucellosis, contraceptive and brucellosis,

There has been no research published investigating the effects of contraception on shedding or *Brucella* infection in animals

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive vaccine in female *Brucella abortus*-positive bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Null Hypotheses:

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

9. Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

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

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

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 2013/2014-2018/2019). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

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

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

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

At the end of the study, all seropositive animals will be euthanized and necropsied with specimens collected for histopathologic, bacteriologic, and molecular analysis. These will include: lymph nodes (bronchial, hepatic, internal iliac, popliteal, mandibular, parotid, prescapular, medial retropharyngeal, and supramammary), mammary gland tissue, spleen, lung, liver ovaries, uterus, cervix, adrenal glands, pituitary gland, and vaccination sites. Vaccinated cows will be euthanized in the chute via captive bolt and exsanguination or high-powered rifle. Alternatively they will be sedated, followed up with captive bolt and exsanguination. The carcasses of animals that have not been vaccinated with GonaCon will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

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

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

Year	Spring	Summer	Fall	Winter
2011	Collect bison for 1st replicate			
2012	Collect bison for 1st and 2nd replicate	Vaccination	Preg check	Preg check
2013	Collect bison for 2nd replicate; Sample collection at calving including culture and serology	Vaccination	Preg check; serology	Preg check serology
2014	Collect bison for 2nd replicate if needed; Sample collection at calving including culture and serology	(Vaccination)	Preg check; serology	Preg check; serology
2015	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2016	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2017	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2018	Sample collection at calving including culture and serology		Preg check; serology	Preg check; serology
2019	(Sample collection at calving including culture and serology)			

10. Experimental Design and Statistical Analyses

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

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

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

12. List of Records to be Maintained

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

13. Cost Estimate for Each Fiscal Year

	FY-12	FY-13	FY-14	FY-15	FY-16	FY-17	FY-18	FY-19
A. Salary and Benefi	$900	$900	$900	$900	$900	$900	$900	$900
B. Facilities	$0	$0	$0	$0	$0	$0	$0	$0
C. Equipment	$0	$0	$0	$0	$0	$0	$0	$0
D. Supplies	$400	$400	$400	$400	$400	$400	$400	$400
E. Animal Care Cost	$0	$0	$0					
F. Operating Costs	$600	$600	$600	$600	$600	$600	$600	$600
TOTAL	$1,900	$1,900	$1,900	$1,900	$1,900	$1,900	$1,900	$1,900

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

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

Jack Rhyan is a veterinarian and pathologist. Dr. Rhyan has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Pauline Nol is a veterinarian. Dr. Nol has 8 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation euthanasia, and necropsy.

Matt McCollum is a wildlife biologist. Mr. McCollum has 10 year of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

Patrick Ryan Clarke is a veterinarian. Dr. Clarke has over 20 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, ear tagging, palpation, euthanasia, and necropsy.

Rebecca Frey is a wildlife biologist. Ms. Frey has 10 years of experience handling bison in both captive and field settings, including anesthesia, injections, blood collection, euthanasia, and necropsy.

16. Archiving

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

17. Protocol Amendments

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

18. References

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

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

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

with GnRH vaccine. Am J Reprod Immunol. 44: 266-74..

Miller, L. A., J. P. Gionfriddo, K. A. Fagerstone, J. C. Rhyan, and G. J. Killian. 2008. The single-shot GnRH immunocontraceptive vaccine (GonaCon) in white-tailed deer: comparison of several GnRH preparations. Am J Reprod Immunol. 60: 214-23.
Miller, L. A., J. C. Rhyan, and M. Drew. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 40: 725-30

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

Robison, C. D. D. S. Davis, J. W. Templeton, M. Westhusin, W. B. Foxworth, M. J. Gilsdorf, L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. J Wildl Dis. 34:582-9.
Yoder, C. A. and L. A. Miller. 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine. 29: 233-9.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

A). **Animal Information**:

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

B1) Rationale for involving animals:

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

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

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

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

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

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

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

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

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

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

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

Reversal for narcotics:
Naltrexone-50 mg IM per mg A3080 given or 100 mg IM per mg carfentanil given

Tolazoline-300 mg as needed IM

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

J) Dietary contaminant exposure NA

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

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

L) Animal pain or distress

L1)Consultation with Attending Veterinarian:
Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Name of Attending Veterinarian: ___ Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

c) Surgery:

M) Euthanasia

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

N. IACUC Approval

Date of IACUC Approval Letter:__ACUC Protocol approved 5/17/2011_See attached____

Bison Quarantine Facility Institutional Animal Care and Use Committee

O. Staff Qualifications

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

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

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

Will this activity occur anyway even without involvement by NWRC?

☒ No

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

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

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

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

This study will have no impact on nontarget species

Effects on T&E species and eagles:

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

☒ No

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

Consultations:

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

☐ No

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

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

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

☐ No, permission not needed because:

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

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

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

D. Route of administration

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

E. Dosage

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

F. Test, control, and reference substance accountability

BT 016.02 Manufacture of GonaCon Immunocontraceptive Vaccine

SOP AD 12.03

G. Material verification

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

ACP Consultation:

USDA

United States Department of Agriculture

Animal and Plant Health Inspection Service

Wildlife Services

National Wildlife Research Center

4101 La Porte Ave.
Ft. Collins, CO
80521

Ph: 970 266-6000
Fax: 970 266-6032

Date: February 21, 2012

To: Jack Rhyan
Study Director

Subj: **NWRC IACUC Deferral Letter for the Approval of Protocol QA-1858**
"Evaluation ofGonaCon™, an immunocontraception vaccine, as a means of decreasing shedding of Brucella abortus in bison."

As Chairperson of the NWRC Institutional Animal Care and Use Committee (IACUC), I have reviewed this proposed study protocol. The Animal Welfare Act requires research activities which use animals for purposes of research, testing, or teaching to be reviewed and approved by the institution's IACUC. The institution's IACUC has direct oversight over the care and use of the animals at that facility or during their research activities. However, when studies are joint collaborations between two or more entities, and/or when activities have been reviewed and approved by the another IACUC, then NWRC IACUC oversight does not need to be duplicated when the other institution is responsible for the IACUC requirements of that activity.

This proposed activity will occur at the Bison Quarantine Feasibility Study Location in Gardiner MT, and has been reviewed and approved by that facility's IACUC (approval dated 5/16/11). Therefore, as Chair of the NWRC IACUC, I hereby defer the approval of this protocol and any proposed changes to the Bison Quarantine Facility's IACUC, who in turn will ensure the proper oversight for the care and use of animals in this study.

If you have any questions or concerns, please contact me at (970) 266-6169 or at steven.j.greiner@aphis.usda.gov. Thank you.

Steve Greiner
Chairperson, NWRC IACUC

GonaCon Study

Research Animals inventory December 13, 2011

	BANGLE TAG	EARTAG	BACKTAG	DATE Rec'vd	Sero-stat	Age/DOB	SEX	Preg?	BLED	OLD EARTAG
	Green 13	81AJW3732		4/26/2011	NEG	0, 2011	F			
	Green 16	81AJW3751		4/26/2011	NEG	0, 2010	F			
	Green 04	YNP930625	81VJ6443	3/8/2011	NEG	2, 2009	F			
	Green 10	YNP930626	81VJ6444	3/8/2011	NEG	2, 2009	F			
	Green 17	YNP930627	81VJ6445	3/8/2011	NEG	2, 2009	F			
	Green 18	YNP930631	81VJ6449	3/8/2011	NEG	1, 2010	F			
	Green 15	YNP930634	81HL6013	3/8/2011	NEG	1, 2010	F			
	Green 07	YNP930638	81HL6017	3/8/2011	NEG	1, 2010	F			
	Green 08	YNP930648	81HL6028	3/8/2011	NEG	2, 2009	F			
	Green 12	YNP930670	81VJ6455	3/10/2011	NEG	1, 2010	F			
	Green 11	YNP930675	81VJ6460	3/10/2011	NEG	2, 2009	F			
	Green 05	YNP930696	81VJ6481	3/10/2011	NEG	1, 2010	F			
	Green 02	YNP930702	81VJ6487	3/10/2011	NEG	1, 2010	F			
	Green 14	YNP930725	81VJ6512	3/10/2011	NEG	2, 2009	F			
	Green 03	YNP930731	81VJ6518	4/5/2011	NEG	1, 2010	F			
	Green 01	YNP930740	81VJ6527	4/5/2011	NEG	1, 2010	F			
	Green 06	YNP930754	81VJ6541	4/5/2011	NEG	1, 2010	F			
	Green 09	YNP930755	81VJ6542	4/5/2011	NEG	1, 2010	F			
	Red 14	YNP930150		4/26/2011	POS	1, 2010	F			
	Red 26	YNP930202		4/26/2011	POS	2, 2009	F			
	Red 06	YNP930287		4/26/2011	POS	1, 2010	F			
	Red 29	YNP930406		4/26/2011	POS	2, 2009	F			
	Red 27	YNP930454		4/26/2011	POS	2, 2009	F			
	Red 01	YNP930472		4/26/2011	POS	1, 2010	F			
	Red 10	YNP930502		4/26/2011	POS	1, 2010	F			
	Red 30	YNP930568		4/26/2011	POS	1, 2010	F			
	Red 28	YNP930575		4/26/2011	POS	2, 2009	F			
	Red 17	YNP930588		4/26/2011	POS	1, 2010	F			
	Red 24	YNP930636		4/26/2011	POS	2, 2009	F			
	Red 23	YNP930667	81VJ6452	3/10/2011	POS	2, 2009	F			

GonaCon Study

Research Animals inventory December 13, 2011

	BANGLE TAG	EARTAG	BACKTAG	DATE Rec'vd	Sero-stat	Age/DOB	SEX	Preg?	BLED	OLD EARTAG
32	**Red 22**	YNP930673	81VJ6458	3/10/2011	POS	2, 2009	F			
33	**Red 20**	YNP930678	81VJ6463	3/10/2011	POS	2, 2009	F			
34	**Red 16**	YNP930684	81VJ6469	3/10/2011	POS	1, 2010	F			
35	**Red 03**	YNP930689	81VJ6474	3/10/2011	POS	2, 2009	F			
36	**Red 05**	YNP930697	81VJ6482	3/10/2011	POS	1, 2010	F			
37	**Red 15**	YNP930706	81VJ6492	3/10/2011	POS	1, 2010	F			
38	**Red 13**	YNP930737	81VJ6524	4/26/2011	POS	1, 2010	F			
39	**Red 04**	YNP930759	6048	5/23/2011	POS	2, 2009	F			
40	**Red 09**	YNP930760	6049	5/23/2011	POS	0, 2011	F			
41	**Red 08**	YNP930761	6050	5/23/2011	POS	2, 2009	F			
42	**Red 19**	YNP930762	8523	5/23/2011	POS	1, 2010	F			
43	**Red 21**	YNP930763	8526	5/23/2011	POS	2, 2009	F			
44	**Red 12**	YNP930765	8528	5/23/2011	POS	1, 2010	F			
45	**Red 07**	YNP930773	8536	5/23/2011	POS	2, 2009	F			
46	**Red 18**	YNP930776	8540	5/23/2011	POS	2, 2009	F			
47	**Red 11**	YNP930777	8541	5/23/2011	POS	1, 2010	F			
48	**Red 25**	YNP930778	8542	5/23/2011	POS	2, 2009	F			
49	**Red 02**	YNP930705	81VJ6491	3/10/2011	SUS	1, 2010	F			

GonaCon Study

Research Animals inventory December 13, 2011

	Datechngd	Disposition	Deworm
		calf of Grn 11	
		Calved	

Research Animals inventory December 13, 2011

	Datechngd	Disposition	Deworm
32			
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United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities*. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation and approval (IACUC, Biosafety, NEPA, ESA) as applicable. ☐ Attach the NWRC Material Transfer Agreement [Standard Form (for intellectual property), Chain of Custody, or Animal/Animal Tissue Transfer Form, as applicable]	Examples: • Activities requiring the use of animals, such as service on student Advisory Committees resulting in authorship, specific training programs, etc. • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the collaborating institution's appropriate regulated documentation and approval (IACUC, Biosafety, NEPA, ESA, MTA/CoC) as applicable.	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.
4 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) ☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation and approval (IACUC, Biosafety, NEPA, ESA, MTA/CoC) as applicable.	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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____
(signature)

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
A. Animal Use		
☐	☐	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.
B. Microbiological/Biohazardous Materials		
☐	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix**.
C. Permits		
☐	☐	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent 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. _____ _____ _____ _____ _____ _____ Permit(s) description Number Date
D. National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☐	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☐	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix**. Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☐	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☐	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
E. Regulatory Standard and Test Guidelines		
☐	☐	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☐	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☐	☐	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
F. Test, Control and Reference Material/Devices		
☐	☐	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
G. Historical Resources		
☐	☐	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.
H. Material Transfer Agreement /Chain of Custody		
☐	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **MTA or CoC Appendix**.
I. Analytical Chemistry		
☐	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix**.

PART THREE: DESCRIPTION OF ACTIVITIES

A. Nature of the collaboration:

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

B. Collaboration:

Name	Address or Organization	Role in Project

C. Start Date:

End Date:

Archive Date:

D. Anticipated Project Outcome:

- ☐ Manuscript
- ☐ Report
- ☐ Other: _____

E. Materials to be archived to close this activity:

F. Description of Project and NWRC Activities and Participation:

G. Comments:

H. 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. 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).

16. Archiving

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

17. Protocol Amendments

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

18. References

List in alphabetical order by author.

19. Appendices

Indicate none or check attached appendices:

☐ None
☐ Animal Use Appendix
☐ Analytical Chemistry Appendix
☐ Column E Explanation
☐ Material Transfer Agreement/Chain of Custody

☐ 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: _____

Analytical Chemistry Appendix

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

A. Number of samples to be analyzed (by type):

B. Storage conditions (temperature, container type, light/dark, duration):

C. Method title and number:

D. ACP Leader approval: _____ Date: _____
(attach email or letter of concurrence from Analytical Services Project Team Leader)

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

Column E Explanation

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

3. Species (common name) of animals used in study during this reporting period:

4. Explain procedure producing pain and/or distress:

5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. The explanation should be scientific in nature, yet easily comprehensible to an educated lay person. (For federally mandated testing, see item 6 below):

6. What, if any, federal regulations require this procedure?

   Agency: CFR:

Material Transfer Agreement

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

PARTIES:

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

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

PURPOSE:

To provide Recipient with _____ 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 shall assume sole responsibility for any claims or liabilities which may arise as a result of the Recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal and Plant Health Inspection Service, the Center for Disease Control, and /or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.

7. APHIS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

8. Upon completion of the activities performed using the Material, the Material shall be returned, destroyed or otherwise disposed of as instructed by APHIS.

9. Recipient shall meet with U.S. Department of Agriculture representatives to determine inventorship if an invention should arise from work with the Material.

10. Recipient shall not disclose Material marked “Confidential” or “Proprietary” to any third party without written permission from APHIS.

11. Material shall be excluded from the confidentiality requirements of this Agreement if: (1) Recipient had possession of the Material prior to disclosure; (2) the Material is generally available to the public at the time of disclosure; (3) the information becomes generally available to the public through no fault of Recipient after disclosure; or (4) after disclosure, Recipient receives the Material from a third party having the right to the Material and who does not impose a confidentiality obligation upon Recipient.

12. If the parties hereto decide, at some future date, to engage in a cooperative research project or program using the Material, a formal Cooperative Research and Development Agreement, or other research Agreement, must be negotiated and entered into between the parties. Such an Agreement shall supersede this Material Transfer Agreement.

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

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

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

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:

Typed name	Signature (NWRC Scientist)	Date
Typed Name	Signature (NWRC Project Leader)	Date

APHIS REVIEWING OFFICIAL:

Typed Name	Signature (NWRC Technology Transfer Program Manager)	Date

APHIS APPROVING OFFICIAL:

Typed Name	Signature (NWRC Assistant Director)	Date

ACCEPTED FOR RECIPIENT:

Typed Name/Title	Signature	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
National Wildlife Research Center

PARTIES:

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

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

PURPOSE:

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

[Table may be adjusted as needed]

Type	Number	ID	Source

The Material is released to Recipient under the following conditions:

1. The Material shall only be used for [give the specific purpose(s) that the material may be used for].

2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of APHIS. Any third party requesting a sample shall be referred to APHIS.

3. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from APHIS.

4. Recipient shall keep APHIS informed of the results obtained through your use of the Material, shall provide APHIS with any manuscript that describes the work with the Material and shall acknowledge APHIS' contribution to the work reported when appropriate.

5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.

6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the

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

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

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

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

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

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

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

ACCEPTED FOR THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE:

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

ACCEPTED FOR RECIPIENT:

Typed Name/Title	Signature	Date

Original: Quality Assurance Unit
CC: Technology Transfer Program Manager

FROM: National Wildlife Research Center Scientist Name Scientist Address City, State Zip Tel: Telephone # of Scientist FAX: FAX # of Scientist E-Mail: E-mail address of Scientist	**TO:** Name Address Tel: FAX: E-Mail:
Materials to Transferred	
Description of Materials: *15 Live Coyotes (see attached sheets (2pages) for details)* *or* *Live coyotes as follows:* *Eartag 15, male, dob 5/12/2006, last rabies shot 1/15/2011* *Eartag 43, female, dob 4/27/07, last rabies shot 1/15/2011* or Blood spots (Whitman spot) for the following coyotes: *Eartag 15, male, dob 5/12/2006, last rabies shot 1/15/2011* *Eartag 43, female, dob 4/27/07, last rabies shot 1/15/2011*	
Purpose: To be analyzed for genetic type	
Final Disposition: e.g., dispose appropriately, incinerate, return to……	
Warranties and Safety: NWRC gives no warranties or guarantees, expressed or implied, for the material, including merchantability or fitness for a particular purpose. Furthermore, NWRC gives no warranties the material is free of pathogens or disease. This material may be infected with pathogens including, but not limited to, [name of pathogen]. Recipient agrees to use materials in accordance with local, state and federal laws governing the use and disposal of these pathogens.	
Statements of Understanding: The Material shall only be used for the purpose stated above. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of NWRC. Any third party requesting a sample shall be referred to NWRC. The Material shall not be used for commercial or profit making purposes without an appropriate license or other permission from NWRC. Recipient shall keep NWRC informed of the results obtained through your use of the Material, shall provide NWRC with any manuscript that describes the work with the Material and shall acknowledge NWRC's contribution to the work reported when appropriate. 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.	
Other Considerations:	
For NWRC Internal Purposes only: Typed name _____ Signature (NWRC Scientist) _____ Date _____ Typed Name _____ Signature (NWRC Project Leader) _____ Date _____ Original: Quality Assurance Unit CC: Technology Transfer Program Manager	

Shipping Information	
Date shipped:	**Method shipped:** *(e.g., FedEx, tracking number######)*
Number/type of shipping containers:	**Shipped by/telephone:**
Comments or Instructions: *(eg. Refrigerate immediately upon arrival, return internal HOBO#1 to: xxx, retain HOBO#2 with samples, contact shipper if samples arrive in broken condition, etc.)*	
Receiving Information	
Date received:	**Received by/telephone:**
Condition of containers and materials upon receipt:	**Were all materials satisfactorily received? If not , please explain:**
Other receiving comments:	
Upon receipt, FAX this completed sheet to: **Attn:** **FAX #:**	

Microbiological/Biohazardous Materials Use Appendix

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

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

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

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

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

B. Is this a Select Agent (see www.selectagents.gov/agentToxinList.htm)?

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

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

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

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

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

G. Permit information:

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

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

Agent Hazards:

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

Laboratory Procedure Hazards:

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

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

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

Biosafety, Security and Additional Precautions:

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

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

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

2. Biosecurity: Describe the procedures, processes, facility controls and equipment that will be used to ensure biosecurity including, 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.

A. 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 <u>not</u> qualify for a Categorical Exclusion.

B. 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.

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

D. Address the potential to impact <u>non-target</u> 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:

E. 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:

☐ Other: *Highly unlikely (risk is negligible) because ……..*

Consultations:

F. 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:

G. 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

☐ Other: *Permission will be obtained prior to entering property…..*

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 ACP Formulation Chemist, complete the following:

ACP Formulation Chemist 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: _____

P.1 Cathy Ben's Meeting Notes

7/5 PUL

mark — telework — email + phone call must be answered.
- forward phone
- leave phone # on Board

GALE — VIDEO & UTUBE
Internet, UCDavis, tv hallways, Kiosk, career fair,

↓

6/7/11 EUP / Brian
N 130 acres
> public potential
Buffalo FireBed Company
SEIS
N legal inquiry

Y EPA Consultation —
, EUP? vs. John — Kim Neshi — KT/ME

Y NEPA EA ← Routine measures ?
req, permiting, public announcement
Feb/March 2012 Stephie Stevens / Annie Manser

Captured outside Park
few more next Spring

p.2 Cathy Ben's Meeting Notes

protocol → Efficacy

talk to Dan Baker

↑

7/11/11 Jeff Kemp

- 7/12/11 orig. formulation BT 016.02

Blue

Sulfo-SMCC 9.5

KLH

✓ QC #1 dialysis

✓ QC #2

dev. Ellman's reagent made w/ PBS no impact

explanation of type of GNRH

✓ QC # 4

Bens, Catherine M - APHIS

From: Bens, Catherine M - APHIS
Sent: Thursday, February 16, 2012 4:39 PM
To: Rhyan, Jack C - APHIS; Nol, Pauline - APHIS
Cc: Greiner, Steven J - APHIS; Laura B Greiner (laura.b.greiner@aphis.usda.gov)
Subject: QAU review protocol QA-1858

Hello Jack/Pauline,

The QA Unit has reviewed your recently submitted protocol and has minor comments below requiring your attention.

Please contact me with any questions or comments.

Cat

****

QA-1858: Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison

NWRC Protocol Classification: 4

GLP Classification: This study is described as a non-regulated with regard to current QA and GLP regulations. It will not require an inspection by the QAU.

COMMENTS:

PART ONE
Signature page: Please obtained new signatures for Jack once comments have been addressed.

PART TWO
Material Transfer Agreement: Biological materials submitted to outside labs, especially MVDL, will need a Material Transfer Agreement. Please check yes and attach a MTA or indicate that a MTA will be developed as part of study conduct.

PART FOUR:

Section 3. Sponsor: Please complete the contract no information. If there is no contract number indicate "NA" or equivalent.

Section 4, Schedule: As Pauline and I discussed, please change the experimental start date to May 2012 as the date test material is applied to test system. Pre-study quarantine are not considered part of this study to my knowledge.

Section 11. SOPs: Generally SOPs included are study specific rather than general NWRC SOPS. Consider removing AD001 and AD002 from this list especially since this study will not be monitored by the QA Unit.

Section 15. Staff Qualification: The section on Dr. Clarke appears to need some editing.

NEPA: In the section marked 'This study does not qualify for a Categorical Exclusion. Please provide information on the EA that is in development.

Catherine Bens
Quality Assurance Manager
USDA/APHIS/WS National Wildlife Research Center
Fort Collins, CO 80521
Phone (970) 266.6053
Cell (970) 214.8035
Fax (970) 266.6010
Catherine.m.bens@aphis.usda.gov

Bens, Catherine M - APHIS

From: Bens, Catherine M - APHIS
Sent: Friday, February 17, 2012 1:53 PM
To: Nol, Pauline - APHIS
Subject: RE: New draft of QA1858

Thanks Pauline. Your changes look fine. Please provide a copy of the EA to Laura for inclusion in our supporting files as soon as it is available.

Cat

From: Nol, Pauline - APHIS
Sent: Friday, February 17, 2012 9:24 AM
To: Greiner, Laura B - APHIS; Bens, Catherine M - APHIS; Greiner, Steven J - APHIS
Subject: New draft of QA1858

I'll have a new signature page for you today.

Thanks!

Pauline

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Office: 970-266-6126
Cell: 970-218-1418
Fax: 970-266-6157

Bens, Catherine M - APHIS

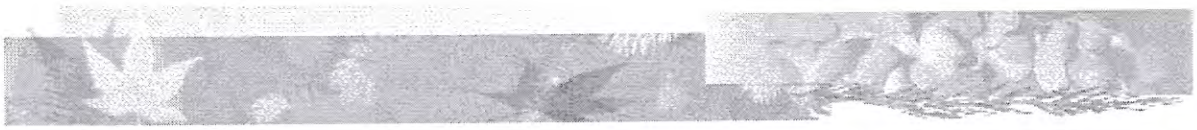
From: Bens, Catherine M (APHIS)
Sent: Wednesday, June 29, 2011 11:49 AM
To: Nol, Pauline (APHIS)
Subject: RE: Meeting about GonaCon Bison Project

All,

I am not available Friday, but tomorrow morning and next week look good.

Cat

From: Nol, Pauline (APHIS)
Sent: Wednesday, June 29, 2011 10:11 AM
To: Bens, Catherine M (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: Meeting about GonaCon Bison Project



Hi Cat and Kathy,

When would you both be available to talk about the best way to develop the NWRC protocol for our bison GonaCon study in Montana?

I'm available tomorrow and Friday and most of next week.

Thanks,

Pauline

****
****
Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

Bens, Catherine M - APHIS

From: Bens, Catherine M - APHIS
Sent: Monday, October 17, 2011 11:12 AM
To: Nol, Pauline - APHIS
Subject: Protocol Template attached no problem (Cut appendix out as needed) eom
Attachments: AD003-05 TEMPLATE complete protocol and all appendices 10-17-11.docx

Catherine Bens
Quality Assurance Manager
USDA/APHIS/WS National Wildlife Research Center
Fort Collins, CO 80521
Phone (970) 266.6053
Cell (970) 214.8035
Fax (970) 266.6010
Catherine.m.bens@aphis.usda.gov

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Wednesday, July 20, 2011 12:00 PM
To: Eisemann, John D (APHIS); O'Hare, Jeanette R (APHIS); Jones, Jeffery W (APHIS)
Subject: FW: GonaCon and Bison Letter
Attachments: GonaCon Bison Letter to EPA.pdf

FYI, a paper copy of the attached letter was delivered to EPA Document Processing this morning. I also sent it electronically to Meredith Laws and John Hebert on Monday. Hopefully we'll hear something on this soon.

When Ann gets back from vacation, she'll send her usual copy to the NWRC archives.

United States
Department of
Agriculture

Marketing and
Regulatory
Programs

Animal and
Plant Health
Inspection
Service

Policy and Program
Development

Environmental and
Risk Analysis
Services, Unit 149
4700 River Road
Riverdale, MD
20737

ENQL 7-1 CY11
PERMANENT
Retire 07/16

July 19, 2011

Meredith Laws
Insecticide-Rodenticide Branch
Registration Division (7504P)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Ave. N.W.
Washington, DC 20460

SUBJECT: **Proposed Study on GonaCon™ (EPA Reg. No. 56228-40) in Bison**

Dear Ms. Laws:

The United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA APHIS VS), in cooperation with USDA APHIS Wildlife Services (WS), plans to conduct a multi-year study in Montana beginning in early 2012 on bison to investigate the efficacy of GonaCon™ as a contraceptive option to potentially assist in controlling the spread of bovine brucellosis.

Brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is found in bison in the Yellowstone basin, including the area in Montana where the planned study will be conducted.

Because brucellosis can be spread through spontaneous abortion or calving of infected female bison, use of a contraceptive has the potential to limit the spread of the disease. GonaCon™, if demonstrated to be efficacious in preventing pregnancy in female bison confirmed to be infected with brucellosis, would be a nonlethal method of potentially decreasing the prevalence of brucellosis in bison.

While some efficacy testing of GonaCon™ in bison has been conducted in very limited studies, additional information on the efficacy in larger groups of animals is important for further evaluation of GonaCon™ as a contraceptive option. The planned study, details of which are discussed below, would gather this needed additional information.

GonaCon™ Study Details

Study Title:	Evaluation of GonaCon™, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison
Number of Study Animals:	96 female bison (24 tested seronegative for brucellosis, 72 tested seropositive for brucellosis) and 4-8 male bison, all tested seronegative for brucellosis
Study Location:	4 contained pens on private leased land in Gardiner, Montana Total area of pens ~100 acres
Pen Fencing:	Double-fenced; outer fences are 8 feet high tensile woven wire, interior fences are 5 feet 7 inch high strand woven wire with electricity, inner and outer fences separated by a space of 10 feet
GonaCon™ Dose Per Animal:	3.0 ml (containing 3000 micrograms active ingredient in 3 ml adjuvant)
Total Number of Animals Receiving GonaCon™:	20 to 24
Maximum Amount Of GonaCon™ Used In Study:	72 ml (containing 72000 micrograms (72 milligrams) active ingredient)

Regulatory and permitting requirements outside of FIFRA, including but not limited to, National Environmental Policy Act (NEPA) requirements, and applicable state and local permits, will be addressed as needed in order to conduct the study. However, because GonaCon™ is currently registered by EPA for use on deer as a contraceptive (EPA Reg. No. 56228-40), APHIS has been carefully considering what approvals for conducting the above study may be required under EPA FIFRA regulations outlined in the Code of Federal Regulations (CFR) at 40 CFR §172 – Experimental Use Permits.

The bison from this proposed will not be consumed. In addition, this planned study will be conducted in secure penned areas as described above. Based on our interpretation of the language in 40 CFR §172.3 (c)(1), we believe an Experimental Use Permit under FIFRA does not need to be obtained.

Because of the scrutiny of bison-related work in Montana, USDA APHIS requests that EPA review the details of the proposed study provided here and confirm in writing that an EUP will not be required.

If you have any questions about this request, please contact Stephanie Stephens by phone at (435) 658-5134 or by e-mail at stephanie.h.stephens@aphis.usda.gov.

Sincerely,

Kenneth R. Seeley, Ph.D.
Chief, Environmental and Risk Analysis Services

cc:
N. Freeman, USDA, APHIS, WS, NWRC Archives, Fort Collins, CO
S. Floyd, USDA, APHIS, PPD, ERAS, Riverdale, MD

File: Section 3 GonaCon FY2011

APHIS:PPD:ES:SStephens:an:435-658-5134:07-18-11: I:\PPD\ES\DataSupport\WS\Pesticides\GonaCon\GonaCon Bison EUP Request.docx

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Thursday, July 14, 2011 11:30 AM
To: Rhyan, Jack C (APHIS)
Cc: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: GonaCon Bison - Letter to EPA
Attachments: Bison GonaCon - Letter to EPA.docx

Hi Jack-As John and I have both discussed with you, we've drafted a letter to EPA to discuss the planned GonaCon bison study, present our rationale for not obtaining and Experimental Use Permit, and request EPA's agreement to this approach in writing.

Attached for your review is the text of the letter that John and I propose submitting to EPA. I'd like to finalize this tomorrow if possible.

Please let me know if you have any changes to it or questions about what we've said.

If you'd like to discuss it in more detail, don't hesitate to call me at 435-658-5134.

Thanks,

Stephanie

Proposed Study on GonaCon in Bison

USDA APHIS Veterinary Services (VS), in cooperation with APHIS Wildlife Services (WS), plans to conduct a multi-year study in Montana beginning in early 2012 on bison to investigate the efficacy of GonaCon as a contraceptive option to potentially assist in controlling the spread of bovine brucellosis.

Brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is found in bison in the Yellowstone basin, including the area in Montana where the planned study will be conducted. Because brucellosis can be spread through spontaneous abortion or calving of infected female bison, use of a contraceptive has the potential to limit the spread of the disease. GonaCon, if demonstrated to be efficacious in preventing pregnancy in female bison confirmed to be infected with brucellosis, would be a nonlethal method of potentially decreasing the prevalence of brucellosis in bison.

While some efficacy testing of GonaCon in bison has been conducted in very limited studies, additional information on the efficacy in larger groups of animals is important for further evaluation of GonaCon as a contraceptive option. The planned study, details of which are discussed below, would gather this needed additional information.

Comment [shs1]: My opinion is that we should not give EPA the protocol for the study so I summarized some of the protocol details, plus other information on fencing not in the protocol, below

Study Title:	Evaluation of GonaCon, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison
Number of Study Animals:	96 female bison (24 tested seronegative for brucellosis, 72 tested seropositive for brucellosis) 4-8 male bison, all tested seronegative for brucellosis
Study Location:	4 contained pens on private leased land in Gardiner, Montana Total area of pens ~100 acres
Pen Fencing:	Double-fenced; outer fences are 8 feet high tensile woven wire, interior fences are 5 feet 7 inch high strand woven wire with electricity, inner and outer fences separated by a space of 10 feet
GonaCon Dose Per Animal:	3.0 ml (containing 3000 micrograms active ingredient in 3 ml adjuvant)
Total Number of Animals Receiving GonaCon:	20 to 24
Maximum Amount Of GonaCon Used In Study:	72 ml (containing 72000 micrograms (72 milligrams) active ingredient)

Comment [shs2]: Per the protocol, there are up to 4 pens in 3 separate locations: Corwin Springs, Riglers pasture (3 miles north) and Slip and Slide (another ½ mile north), not sure if we should list the specific locations here or not

Comment [shs3]: I have heard estimates of the total study area acreage from 100 to 150, but the protocol says up to 4 pens of about 23 acres each so I'm not sure which total to include, I chose the lower number

Because GonaCon is currently registered by EPA for use on deer as a contraceptive (EPA Reg. No. 56228-40), APHIS has been carefully considering what approvals for conducting the above study may be required under EPA FIFRA regulations. Other regulatory and permitting requirements outside of FIFRA, including but not limited to National Environmental Policy Act (NEPA) requirements, and applicable state and local permits, will be addressed as needed in order to conduct the study. However, APHIS believes that because this planned study will be conducted in secure penned areas as described above, an Experimental Use Permit under FIFRA does not need to be obtained.

Because of the scrutiny of bison-related work in Montana, USDA APHIS requests that EPA review the details of the proposed study provided here and confirm in writing that an EUP will not be required.

If you would like to discuss this request or the proposed study in more detail, please do not hesitate to contact Stephanie Stephens at ....(etc)

Eisemann, John D - APHIS

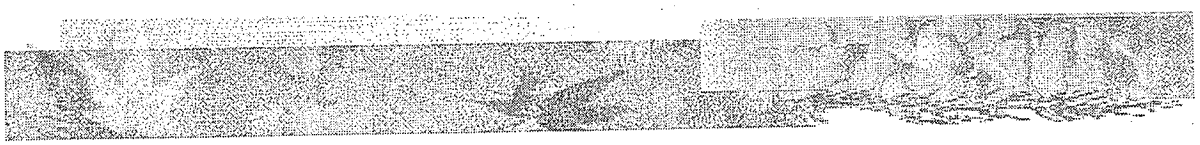
From: Rhyan, Jack C (APHIS)
Sent: Friday, July 08, 2011 1:35 PM
To: Eisemann, John D (APHIS); Stephens, Stephanie H (APHIS)
Subject: FW: amendment document for the IACUC
Attachments: ACUC Proposal GonaConBisonStudy2011amendmentform7.1.11.docx; Immunocontraceptive Bison Protocol.docx

John and Stephanie,

Here are the amendments including the 3rd objective about efficacy. I also attached the previous protocol.

Jack

From: Nol, Pauline (APHIS)
Sent: Friday, July 01, 2011 2:47 PM
To: Rhyan, Jack C (APHIS)
Subject: amendment document for the IACUC



This will be attached to the original document after approval.

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

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

Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Jason Lombard	USDA, APHIS, VS	Investigators
Rick Wallen, Jenny Powers	National Park Service	Investigators
Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, National Wildlife Research Center	Investigators

Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to calves through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus.*

Assurance of Non-Duplication of Studies
Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

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

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

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-

2017). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). During the abortion/calving seasons (from February until August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Within five days of abortion/parturition, the cow will be darted and blood, milk, and vaginal swabs will be collected for serology and culture. If possible, the calf will be captured and conjunctival swabs will be collected for culture and blood collected for culture and serology.

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

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

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

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

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

Experimental Design and Statistical Analyses

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

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

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

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

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

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

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

Animal pain or distress

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

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

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

Amendments:

DESCRIPTION OF ACTIVITIES

The end date to this project should be changed to October 1, 2019

STUDY PROTOCOL

2. Testing Facilities

Montana Veterinary Diagnostic Laboratory will also be receiving serum for Brucellosis testing.

7. Objective/Hypotheses

In this section, Major Objective (2) will be added and will deal with evaluating efficacy of GonaCon™. Consequently, an additional hypothesis (2) will be added. The original Major Objective number 3 will be changed to come under the Minor Objectives section.

This section will read as follows:

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the efficacy of GonaCon™ as an immunocontraceptive in female *B. abortus*-infected bison
3. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

8. Methods/Procedures

Serologic testing for anti-GnRH antibodies will also be conducted in this project. The paragraph below will be added to the section.

Serology evaluating antibody production against GnRH will be conducted at the National Wildlife Research Center. Serology will be conducted prior to vaccination and at least annually thereafter.

10. Experimental Design and Statistical Analyses

This section will be changed to add sample size justification in reference to efficacy testing of GonaCon™ to prevent pregnancies in female bison. In addition, we will add the term "shedding" as a response variable in addition to "abortion". This section will read as follows:

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

As we consider power to be acceptable at a level of approximately 80% for evaluating vaccine efficacy, the number of animals involved in this study is appropriate. The vaccine will be deemed successful if the number of births in non-vaccinates exceeds that of vaccinates by 60% or more. Using a power calculation in SAS (power for comparing 2 independent proportions), a sample size of 10 or greater per group was calculated to be sufficient in order to determine efficacy of the vaccine under the above-stated power constraint.

SIGNATURE PAGE

Study Director _____ Date_____

Concur

IACUC Chair _____ Date_____

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Thursday, July 14, 2011 10:58 AM
To: Eisemann, John D (APHIS)
Subject: GonaCon Bison EUP
Attachments: Bison GonaCon - Documentation for Not Obtaining EUP.docx

John-Attached is the first draft of the request to EPA for agreement on not obtaining an EUP. I was trying to strike a balance between providing enough information on the study, while also not complicating matters too much by introducing much about the disease aspect of the study. Please review and edit however you feel is necessary.

Thanks,

Stephanie

Bison GonaCon Study-Justification for Not Obtaining Experimental Use Permit from EPA

USDA APHIS Veterinary Services (VS), in cooperation with APHIS Wildlife Services (WS), plans to conduct a study in Montana on bison to investigate the efficacy of GonaCon as a contraceptive option to potentially assist in controlling the spread of bovine brucellosis.

Brucellosis, a zoonotic bacterial disease caused by *Brucella abortus*, is found in bison in the Yellowstone basin, including the area in Montana where the planned study will be conducted. Because brucellosis can be spread through spontaneous abortion or calving of infected female bison, use of a contraceptive has the potential to limit the spread of the disease. GonaCon, if demonstrated to be efficacious in preventing pregnancy in female bison confirmed to be infected with brucellosis, would be a nonlethal method of potentially decreasing the prevalence of brucellosis in bison.

While some efficacy testing of GonaCon in bison has been conducted in very limited studies, additional information on the efficacy in larger groups of animals is important for further evaluation of GonaCon as a contraceptive option. The planned study, details of which are discussed below, would gather this needed additional information.

Comment [shs1]: My opinion is that we should not give EPA the protocol for the study so I summarized some of the protocol details, plus other information on fencing not in the protocol, below

Study Title:	Evaluation of GonaCon, an immunocontraceptive vaccine, as a means of decreasing shedding of *Brucella abortus* in bison
Number of Study Animals:	96 female bison (24 tested seronegative for brucellosis, 72 tested seropositive for brucellosis) 4-8 male bison, all tested seronegative for brucellosis
Study Location:	4 contained pens on private leased land in Gardiner, Montana Total area of pens ~100 acres
Pen Fencing:	Double-fenced; outer fences are 8 feet high tensile woven wire, interior fences are 5 feet 7 inch high strand woven wire with electricity, inner and outer fences separated by a space of 10 feet
GonaCon Dose Per Animal:	3.0 ml (containing 3000 micrograms active ingredient in 3 ml adjuvant)
Total Number of Animals Receiving GonaCon:	20 to 24
Maximum Amount Of GonaCon Used In Study:	72 ml (containing 72000 micrograms (72 milligrams) active ingredient)

Comment [shs2]: Per the protocol, there are up to 4 pens in 3 separate locations: Corwin Springs, Riglers pasture (3 miles north) and Slip and Slide (another ½ mile north), not sure if we should list the specific locations here or not

Comment [shs3]: I have heard estimates of the total study area acreage from 100 to 150, but the protocol says up to 4 pens of about 23 acres each so I'm not sure which total to include

Because GonaCon is currently registered by EPA for use on deer as a contraceptive (EPA Reg. No. 56228-40), APHIS has been carefully considering what approvals for conducting the above study may be required under EPA FIFRA regulations. Other regulatory and permitting requirements outside of FIFRA, including but not limited to National Environmental Policy Act (NEPA) requirements, and applicable state and local permits, will be addressed as needed in order to conduct the study. However, APHIS believes that because this planned study will be conducted in secure penned areas as described above, an Experimental Use Permit under FIFRA does not need to be obtained.

USDA APHIS requests that EPA review the details of the proposed study provided here and confirm in writing that an EUP will not be required. Because of the scrutiny of bison-related work in Montana, APHIS believes it is necessary to have EPA's agreement on this issue in writing.

If you would like to discuss this request or the proposed study in more detail, please do not hesitate to contact ... (etc...)

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Thursday, July 07, 2011 8:30 AM
To: Eisemann, John D (APHIS)
Subject: RE: Continued discussion about the Bison study

Sounds good. Thanks.

From: Eisemann, John D (APHIS)
Sent: Wednesday, July 06, 2011 5:09 PM
To: Fagerstone, Kathleen A (APHIS); Bens, Catherine M (APHIS); O'Hare, Jeanette R (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: Continued discussion about the Bison study

Can we plan on meeting in the conference room by us at 1:30 pm. Stephanie, I will call you.

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

From: Fagerstone, Kathleen A (APHIS)
Sent: Wednesday, July 06, 2011 4:49 PM
To: Bens, Catherine M (APHIS); Eisemann, John D (APHIS); O'Hare, Jeanette R (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: Continued discussion about the Bison study

I am available before 10 or after Jeanette's seminar but before 2 PM. Or after 3 PM.

From: Bens, Catherine M (APHIS)
Sent: Wednesday, July 06, 2011 4:10 PM
To: Eisemann, John D (APHIS); O'Hare, Jeanette R (APHIS); Fagerstone, Kathleen A (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: Continued discussion about the Bison study

I am available almost any time. Tomorrow is Jeanette's seminar, perhaps right afterward.

Cat

From: Eisemann, John D (APHIS)
Sent: Wednesday, July 06, 2011 4:01 PM
To: O'Hare, Jeanette R (APHIS); Fagerstone, Kathleen A (APHIS); Bens, Catherine M (APHIS); Stephens, Stephanie H (APHIS)
Subject: Continued discussion about the Bison study

I had discussions today with both Kathy and Stephanie about the decision to not conduct this study under an EUP. Both Stephanie and I were under the impression that this issue had been resolved and the study would proceed under an EUP. Stephanie and I both have reservations about not applying for an EUP for the study. Granted it is technically in a pen(s), but the total area is over 120 acres. In addition, everything about VS's work with bison is highly scrutinized by

the public. In fact, Buffalo Fields Campaign has already called EPA directly and asked if it was legal to conduct this study. 000103

Because of the contentious nature of the study and the fact the EPA may not consider a study this size exempt from and EUP, can we have another internal discussion before we proceed. We feel that it is best to get our own ducks in order before we go back to Jack. Stephanie is confident Jack will do whatever we determine to be the best course.

Are people available to have a discussion tomorrow? I am available anytime. Can you let me know your availability?

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Eisemann, John D - APHIS

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Eisemann, John D - APHIS

From: O'Hare, Jeanette R (APHIS)
Sent: Wednesday, July 06, 2011 4:37 PM
To: Eisemann, John D (APHIS)
Subject: RE: Continued discussion about the Bison study

I'm available too – except 11:00 to noon.

From: Eisemann, John D (APHIS)
Sent: Wednesday, July 06, 2011 4:01 PM
To: O'Hare, Jeanette R (APHIS); Fagerstone, Kathleen A (APHIS); Bens, Catherine M (APHIS); Stephens, Stephanie H (APHIS)
Subject: Continued discussion about the Bison study

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T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Wednesday, July 06, 2011 4:15 PM
To: Bens, Catherine M (APHIS); Eisemann, John D (APHIS); O'Hare, Jeanette R (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: RE: Continued discussion about the Bison study

I am also available any time except for 8:30 – 9:30 am MT.

-Stephanie

From: Bens, Catherine M (APHIS)
Sent: Wednesday, July 06, 2011 4:10 PM
To: Eisemann, John D (APHIS); O'Hare, Jeanette R (APHIS); Fagerstone, Kathleen A (APHIS); Stephens, Stephanie H (APHIS)
Subject: RE: Continued discussion about the Bison study

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John.D.Eisemann@aphis.usda.gov

Eisemann, John D - APHIS

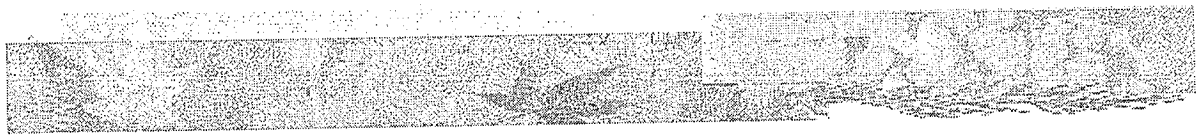
From: O'Hare, Jeanette R (APHIS)
Sent: Friday, July 01, 2011 10:59 AM
To: Fagerstone, Kathleen A (APHIS); Eisemann, John D (APHIS)
Subject: BFC press release on Yellowstone bison/contraception

FYI – in case you have not seen yet. http://www.buffalofieldcampaign.org/media/press1011/pressreleases1011/053111.html

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

Eisemann, John D - APHIS

From: Nol, Pauline (APHIS)
Sent: Monday, June 06, 2011 3:35 PM
To: Miller, Lowell A (APHIS)
Cc: Rhyan, Jack C (APHIS); Eisemann, John D (APHIS)
Subject: filling in gaps in GonaCon Bison Protocol
Attachments: AD003-04 GonaConBisonStudy2011 QA 1858 draft_6 3 11 eisemann commentspnrevision_6.6.docx



Lowell,

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

Thanks,

Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

1.1 **United States Department of Agriculture**

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

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) X☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☒ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix.**
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____National Park Service_____ _YELL-2011-SCI-5892_____ May 10, 2011____ _____ _____ _____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix.** Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: June 2, 2011
☒ ~~☐~~	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☒ ~~☐~~	☐ ~~☒~~	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☒	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement.**
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix.**

Comment [pn1]: ??

PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, ~~2019~~2017

Archive Date: October 10, 2019

Comment [pn2]:

Anticipated Project Outcome:
- ☒ Manuscript
- ☒ Report
- ☐ Other: _____

Materials to be archived to close this activity:
Raw data
Final Report

Description of Project and NWRC Activities and Participation:
~~See research plan~~This study is not part ~~of~~ an NWRC Project.
NWRC's role in this study will be to provide GonaCon and to run ELISAs to determine anti-GnRH titers.

Comments:

Attachments: (e.g. Material Transfer Form, IACUC approval, etc.)	IACUC Protocol Approval Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2017
Proposed Study Completion/Archive Date: October 1, 2019

Comment [pn3]:

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella* abortus, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al.; 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

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

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

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

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The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

9. Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

Comment [jde4]: For the Experimental Use Permit (EUP), you will want to include a map showing the test site location and the layout of the pens (including size).

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

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

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

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

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

Bulls will be separated from the cows outside of breeding season, from October until July. Prior to exposure to bulls, cows will have breeding tags attached to document mounting behavior. Following the first exposure to the bulls in 2012, five calving seasons will be observed (2013-2017 and 20). In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009).

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

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

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

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

Comment [jde8]: NWRC will conduct ELISA tests to determine anti-GnRH titers.

EPA will want to see two measures of efficacy to prove GonaCon will work in bison. This study will actually have more than two measures. 1) pregnancy rates, 2) number of calves produced, 3) anti-GnRH titers.

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

Comment [jde9]: This should be pointed out in the EUP. I want EPA to know you intend to send the animals to slaughter at the end of the study.

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

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

10. Experimental Design and Statistical Analyses

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

A. Protocol and Amendments
B. Correspondence, telephone logs and related records
C. Data records including:
    a. Animal handling and sample collection records
    b. Necropsy records
    c. Results of serologic, histopathologic, and cultural analysis

d.
e.

D. Final Report
E. _____

13. Cost Estimate for Each Fiscal Year

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

Comment [pn10]:

Comment [jde11]: Cost?

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

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

16. Archiving

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

17. Protocol Amendments

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

18. References

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

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

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

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

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

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

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

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

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

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

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

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

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

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

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

This would be an NWRC IACUC type question!

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

Comment [jde13]: Again an NWRC IACUC type question.

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

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

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

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

Butorphenol- 0.03-0.06 mg/kg, IM dart

Medetomidine- 0.01-0.02 mg/kg
Azaperone- 0.02 mg/kg

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

a) Alternative procedures:

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

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

14) Staff Qualifications

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

N. IACUC Approval

Date of IACUC Approval Letter: IACUC Protocol approved 5/17/2011_See attached

Comment [pn14]: By Montana IACUC-Name?

O. Staff Qualifications

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

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

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

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

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

This study will have no impact on nontarget species

Effects on T&E species and eagles:

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

☒ No

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

Consultations:

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

☐ No

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

Comment [pn15]:

Comment [jde16]: You should be able to provide the names for contacts at a number of state and federal entities involved in bison management, particularly those involved in this study.

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:

Comment [pn17]:

☒ Yes

Comment [jde18]: This is the person who manages the corrals where the bison will be kept.

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

GnRH/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml
AdjuVac™ adjuvant	
Mycobacterium avium (Mycopar™ – *M. a. paratuberculosis*)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

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

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

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

D. Route of administration

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

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. ~~Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.~~

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

F. Test, control, and reference substance accountability

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

Comment [pn21]: ??

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

TRCS tracking number(s):_____

G. Material verification

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

Comment [pn23]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:
ACP Consultation: _____ Date: _____

Eisemann, John D - APHIS

From: Nol, Pauline (APHIS)
Sent: Monday, June 06, 2011 1:45 PM
To: O'Hare, Jeanette R (APHIS); Eisemann, John D (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

I hijacked that information from the elk protocol. This can be changed however it needs to be changed.
Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

From: O'Hare, Jeanette R (APHIS)
Sent: Monday, June 06, 2011 1:44 PM
To: Eisemann, John D (APHIS); Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

Just a note to concur with John's comment in the protocol regarding the GonaCon formulation. What you have in the protocol right now is the currently registered product. Lowell has made several changes for a new formulation which have significant regulatory implications. We need to clarify this.

Jeanette

From: Eisemann, John D (APHIS)
Sent: Monday, June 06, 2011 11:03 AM
To: Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS); O'Hare, Jeanette R (APHIS)
Subject: comments on bison protocol

I am around all week if you want to discuss any of these comments.

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

Eisemann, John D - APHIS

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Sent: Monday, June 06, 2011 1:44 PM
To: Eisemann, John D (APHIS); Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

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Fort Collins, CO 80526
T: 970-266-6158
F: 970-266-6157
John.D.Eisemann@aphis.usda.gov

Eisemann, John D - APHIS

From: Nol, Pauline (APHIS)
Sent: Friday, June 03, 2011 3:24 PM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study
Attachments: AD003-04 GonaConBisonStudy2011 QA 1858 draft_6.3.11.docx

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

Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

From: Eisemann, John D (APHIS)
Sent: Friday, June 03, 2011 10:46 AM
To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)
Subject: Meeting to discuss the Bison Study

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

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

1.1 United States Department of Agriculture

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

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) ☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☒ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix.**
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____National Park Service_____ _YELL-2011-SCI-5892_____ May 10, 2011____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix.** Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☐	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☐	☒	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix.** Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☒	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement.**
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix.**

Comment [pn1]: ??

PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date:

Comment [pn2]:

Anticipated Project Outcome:
- ☒ Manuscript
- ☒ Report
- ☐ Other:

Materials to be archived to close this activity: Raw data, Final Report

Description of Project and NWRC Activities and Participation: See research plan

Comments:

Attachments: (e.g. Material Transfer Form, IACUC approval, etc.)	IACUC Protocol Approval Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2019
Proposed Study Completion/Archive Date:

Comment [pn3]:

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella* abortus, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

9. Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

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

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

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

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

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

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

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

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

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

10. Experimental Design and Statistical Analyses

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

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

Comment [pn4]:

13. Cost Estimate for Each Fiscal Year

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

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

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

16. Archiving

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

17. Protocol Amendments

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

18. References

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

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

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

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

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Azaperone- 0.02 mg/kg

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

13) Euthanasia

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

14) Staff Qualifications

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

N. IACUC Approval

Date of IACUC Approval Letter:__IACUC Protocol approved 5/17/2011_See attached____

Comment [pn5]: By Montana IACUC-Name?

O. Staff Qualifications

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

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

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

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

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

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? ☒ 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

Comment [pn6]:

Comment [pn7]:

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

GnRH/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml
AdjuVac™ adjuvant	
Mycobacterium avium (Mycopar™ – *M. a. paratuberculosis*)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

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

D. Route of administration

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

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

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

Comment [pn8]: ??

TRCS tracking number(s):_____

G. Material verification

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

Comment [pn9]: ???

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

ACP Consultation: _____ Date: _____

Eisemann, John D - APHIS

From: Fagerstone, Kathleen A (APHIS)
Sent: Friday, June 03, 2011 11:16 AM
To: Keirn, Gail M (APHIS); Cole, Lyndsay M (APHIS)
Cc: Eisemann, John D (APHIS); Miller, Lowell A (APHIS); Rhyan, Jack C (APHIS); Clark, Larry (APHIS)
Subject: FW: ACTION ITEM - Draft key messages for your review (GonaCon-bison)
Attachments: GonaCon Use in Bison_Key Messages_June 2011.docx; GonaCon Use in Bison_Key Messages_June 2011kf.docx

Gail—I made some edits to the key messages.
Kathy

From: Miller, Lowell A (APHIS)
Sent: Friday, June 03, 2011 10:51 AM
To: Fagerstone, Kathleen A (APHIS); Eisemann, John D (APHIS)
Subject: FW: ACTION ITEM - Draft key messages for your review (GonaCon-bison)

From: Keirn, Gail M (APHIS)
Sent: Friday, June 03, 2011 10:49 AM
To: Cole, Lyndsay M (APHIS); Rhyan, Jack C (APHIS)
Cc: Clark, Larry (APHIS); Miller, Lowell A (APHIS)
Subject: ACTION ITEM - Draft key messages for your review (GonaCon-bison)

Lyndsay and Jack,

Attached are draft messages regarding GonaCon and the proposed bison study. Please review and provide edits to me by **Wednesday, June 22**. I can incorporate everyone's changes and post the final version on the LPA server for future reference by LPA. These bullets will help ensure consistent messaging by VS, WS and LPA.

Thanks,
Gail

Gail Keirn
Public Affairs Specialist
USDA-APHIS
Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone: 970-266-6007
Fax: 970-266-6010

Key Messages

GonaCon Use in Bison

June 2011(Updated 3/1/2012~~6/3/2011~~)

Anticipated media questions:

1. *What is GonaCon?*
2. *How do you know if it will work on bison?*
3. *What did preliminary studies show?*
4. *Is it registered for bison?*
5. *What permits are required to use GonaCon?*
6. *Will GonaCon only be given to female bison? Why not males?*
7. *Does GonaCon have any side effects in bison or other animals?*
8. *If we "eliminate" brucellosis from bison, can't they just get re-infected by elk?*
9. *On what other species has GonaCon been tested?*
10. *How long will the vaccine last? Will the bison need a booster shot?*
11. *Won't sterilizing bison result in the permanent removal of those animals from the gene pool and the creation of a new "unnatural" class of animals?*
12. *How will the animals be monitored and for how long?*
13. *How and when will we know if this experiment is deemed a success?*
14. *What will happen to the bison at the end of study?*
15. *How can I learn more about the study?*

Main Messages

1. **The proposed study will evaluate a new strategy for preventing the spread of brucellosis in bison. Bison brucellosis is transmitted through contact with bodily fluids, such as milk and after-birth tissues, of infected animals. The new immunocontraceptive vaccine— GonaCon™ —could potentially break the cycle of this disease and reduce transmission by preventing reproduction in infected animals. The vaccine is not being used to manage or control overall bison populations.**
2. **An Environmental Assessment (EA) for the proposed study will be available for public comment this fall.**
3. **The bison held at the Corwin Springs facility north of Gardiner, Montana, are being well cared for by APHIS personnel. The National Park Service issued a research permit to APHIS allowing them to hold the bison at the facility.**

Overview of GonaCon

- GonaCon is the first single-shot, multi-year immunocontraceptive vaccine registered for use in mammals. Not only may this new tool be useful as part of urban white-tailed deer management plans where traditional options are limited, but it also shows promise in other areas, such as disease prevention.
- The GonaCon™ Immunocontraceptive Vaccine was officially registered by the U.S. Environmental Protection Agency on September 29, 2009 for use with female white-tailed deer. Its EPA reg. number is 56228-40.

Comment [k1]: SpayVac (a PZP vaccine) is also a single-shot multi-year immunocontraceptive—not registered

- GonaCon has been successfully tested in a variety of mammal species, including deer, elk, feral horses, bison, prairie dogs, ground squirrels, wallabies, and feral dogs and cats.
- In addition to wildlife management research, NWRC and its collaborators' studies include the development of a combined GonaCon-rabies vaccine for use in feral dogs and raccoons, contraception for companion animals, and the prevention of adrenocortical disease in pet ferrets and spread of brucellosis in bison.
- GonaCon is being used for research purposes in the United States, Mexico, Europe, New Zealand and Australia.
- Future NWRC research with GonaCon will likely involve studies to support expanded registration to other species, develop oral delivery systems, and prevent transmission of wildlife diseases.
- GonaCon is registered for white-tailed deer as a restricted-use pesticide, and all users must be Certified Pesticide Applicators. Only USDA Wildlife Services or State wildlife management agency personnel or individuals working under their authority can use it. In order for GonaCon to be used in any given State, it must also be registered with the State and approved for use by the State fish and game/natural resource agency.
- To learn more about NWRC and the development of GonaCon, visit our website at http://www.aphis.usda.gov/wildlife_damage/nwrc/.
- NWRC initiated GnRH reproductive inhibition studies on deer in 1996.

Comment [k2]: Will not be a combined vaccine in one syringe because of different regulatory authorities for the immune and rabies vaccines. Instead will be side-by-side injections. Also, the way this is phrased, it sounds like the combined GonaCon/rabies vaccine will be used as a contraception in companion animals etc.

Comment [k3]: The registration label for use in bison could be different than this.

How Does the Vaccine Work?

- GonaCon stimulates the production of antibodies that bind to GnRH, a hormone in an animal's body that signals the production of sex hormones (e.g., estrogen, progesterone, and testosterone). By binding to GnRH, the antibodies reduce GnRH's ability to stimulate the release of these sex hormones. All sexual activity is decreased, and animals remain in a nonreproductive state as long as a sufficient level of antibody activity is present.
- A decrease in a vaccine's effectiveness is expected as years pass and the number of antibodies towards the vaccine in the animal's body declines.
- Animals can be safely injected with GonaCon more than once in the first year and in subsequent years as the effect of the vaccine wears off.

GonaCon Use in Bison

- Brucellosis is a bacterial disease that causes infertility, abortions, and lowered milk production in cattle, bison, and elk. The disease is transmitted through contact with bodily fluids, such as milk and after-birth tissues, of infected animals. GonaCon could potentially break the cycle of this disease and reduce transmission by preventing reproduction in infected animals.
- APHIS is exploring whether GonaCon could be part of a nonlethal strategy to reduce brucellosis prevalence in bison.
- In 2002, APHIS tested the efficacy of GonaCon in captive female bison. None of the six bison treated with a single dose of GonaCon became pregnant during the subsequent breeding season. Five non-treated bison became pregnant and delivered viable calves. Treated animals remained infertile for at least three years. (*MILLER, L. A., J. C. RHYAN, AND M. DREW. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. Journal of Wildlife Diseases 40:725-730.)*
- In collaboration with the National Park Service, APHIS is proposing a study to evaluate if the infertility of *Brucella abortus*-seropositive female bison (via GonaCon) will decrease the shedding of *Brucella abortus* in a bison herd.

- To ensure statistically significant results, approximately 100 bison will be included in the study. These bison are being housed at the Corwin Springs facility north of Gardiner, Montana.
- GonaCon is currently registered for use in female-white tailed deer, but Experimental Use Permits can be granted by the U.S. EPA to support studies with other species. APHIS has requested an Experimental Use Permit to conduct GonaCon studies to support potential future registration of the vaccine for bison.
- Starting in the spring 2012, animals will be randomly selected for treatment with GonaCon. Animals will be monitored for five subsequent calving seasons (2013-2017). Researchers will be checking animals for pregnancy and abortions, labor, and parturition events, as well as the presence of *B. abortus*.
- Each treatment and control group will include 16-18 *Brucella abortus*-seropositive female bison, 4 negative female bison, and 2 negative bull bison. Each treatment animal will receive one shot of the GonaCon vaccine.
- Only female bison will be treated with GonaCon, as they are the primary transmitters of brucellosis through infected milk and aborted fetuses.
- The health effects associated with GonaCon are minimal. Vaccinated animals showed a decrease in sexual activity and breeding behavior. In pen studies, animals showed little to no visual evidence of inflammation at injection sites, and blood chemistry was similar among treatment and control groups.
- At the completion of the study:
  - All seropositive bison will be euthanized and necropsied with specimens collected for culture. The carcasses will be donated to local food banks or Indian tribes. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.
  - All or a subset of offspring that remain or become seropositive for *B. abortus* after weaning will be maintained and monitored through their first parturition.
  - Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements will be used for bison conservation.
- APHIS and its collaborators continue to explore new methods for eliminating brucellosis in cattle and wildlife. This study will provide valuable information for the development of long-term strategies for reducing the spread of brucellosis in bison.
- To learn more, visit the Interagency Bison Management Plan website at http:\IBMP.info.

Background on WS/NWRC

- USDA Wildlife Services provides federal leadership and expertise to resolve human-wildlife conflicts. WS also works to protect wildlife from adverse human activities while reducing the damage and hazards caused by wildlife.
- The National Wildlife Research Center (NWRC) is the research arm of Wildlife Services.
- Our mission is to develop science-based solutions to resolve wildlife-human conflicts, such as damage to agriculture and livestock, prevention of wildlife diseases, wildlife conflicts at airports, invasive species impacts to native wildlife and habitats.
- NWRC employs about 170 scientists and support staff.
- As part of its scientific efforts, NWRC investigates new tools and methods for use in wildlife damage management.

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Key Messages

GonaCon Use in Bison
June 2011(Updated 3/1/2012)

Anticipated media questions:

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2. *How do you know if it will work on bison?*
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2. **An Environmental Assessment (EA) for the proposed study will be available for public comment this fall.**
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- To learn more, visit the Interagency Bison Management Plan website at http:\IBMP.info.

Background on WS/NWRC

- USDA Wildlife Services provides federal leadership and expertise to resolve human-wildlife conflicts. WS also works to protect wildlife from adverse human activities while reducing the damage and hazards caused by wildlife.
- The National Wildlife Research Center (NWRC) is the research arm of Wildlife Services.
- Our mission is to develop science-based solutions to resolve wildlife-human conflicts, such as damage to agriculture and livestock, prevention of wildlife diseases, wildlife conflicts at airports, invasive species impacts to native wildlife and habitats.
- NWRC employs about 170 scientists and support staff.
- As part of its scientific efforts, NWRC investigates new tools and methods for use in wildlife damage management.

Eisemann, John D - APHIS

From: Miller, Lowell A (APHIS)
Sent: Friday, June 03, 2011 10:56 AM
To: Eisemann, John D (APHIS)
Subject: FW: copy of IACUC for bison GonaCon study
Attachments: ACUCBisonGonaConStudyfinal.pdf; ACUC Comm signaturesGonaConBisonStudy.pdf

---

From: Rhyan, Jack C (APHIS)
Sent: Thursday, June 02, 2011 4:14 PM
To: Miller, Lowell A (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: copy of IACUC for bison GonaCon study

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

REGULATORY CONSIDERATIONS

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

DESCRIPTION OF ACTIVITIES

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

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

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

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

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

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2019

5. Background and Justification

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

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

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

8. Methods/Procedures

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

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

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

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

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

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

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

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

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

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

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

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

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

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

IA.

10. Experimental Design and Statistical Analyses

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

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

11. Animal Care and Use Information

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

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

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

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

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

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

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

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

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

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

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

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

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

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

12. Staff Qualifications

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

13. References

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

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

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

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

SIGNATURE PAGE

Study Director _____ Date 5/16/2011

Concur

IACUC Chair _____ Date _____

PART ONE: SIGNATURE PAGE

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

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

IACUC Committee member [signature] 5/24/11

IACUC Committee member

Return FAX# R. Clarke: 386-5162

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: 5/16/11

Concur:
IACUC Chair _____ Date 5/16/11

IACUC committee member _____ 5/25/11
Jerry Wiscomb

IACUC committee member _____ _____

Return FAX# R Clarke: 388-5162

Eisemann, John D - APHIS

From: Miller, Lowell A (APHIS)
Sent: Friday, June 03, 2011 10:40 AM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: FW: Bison-GonaCon study proposed for Montana

FYI

From: Keirn, Gail M (APHIS)
Sent: Thursday, June 02, 2011 4:18 PM
To: Steuber, John (APHIS)
Cc: Graves, George E (APHIS); Krischke, Rodney F (APHIS); Clark, Larry (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Cole, Lyndsay M (APHIS)
Subject: Bison-GonaCon study proposed for Montana

John,

Just wanted to give you a heads-up that NWRC is providing APHIS Veterinary Services (VS) with samples of the GonaCon vaccine for use in a study with Yellowstone bison.

The proposed VS study will evaluate a new strategy for preventing the spread of brucellosis in bison. As you know, bison brucellosis is transmitted through contact with bodily fluids, such as milk and after-birth tissues, of infected animals. GonaCon could potentially break the cycle of this disease and reduce transmission by preventing reproduction in infected animals. The study is **not** investigating the use of GonaCon to manage or control overall bison populations.

An Environmental Assessment (EA) for the proposed study will be available for public comment this fall. The actual study will likely not begin until the spring of 2012. It will last approximately 5 years, as VS researchers follow the bison through several breeding seasons.

The National Park Service has issued a research permit to VS allowing them to hold bison for the study at the Corwin Springs facility in Gardiner, MT.

VS is still working out the details of this project and would like to keep it low key for now. However, we wanted you to be aware of this potential study in Montana. If you receive questions regarding the study, please refer them to Public Affairs Specialist Lyndsay Cole (301-538-9213) or me to ensure consistent messaging.

Feel free to give me a call when you return to the office and we can discuss further, if necessary.

Regards,
Gail

Gail Keirn
Public Affairs Specialist
USDA-APHIS
Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80521
Phone: 970-266-6007
Fax: 970-266-6010

Eisemann, John D - APHIS

From: Stephens, Stephanie H (APHIS)
Sent: Tuesday, May 31, 2011 1:58 PM
To: Edmundson, Jack P (APHIS); Gutierrez, Vicki L (APHIS); Nasr, Ann M (APHIS); Donch, Debra A (APHIS)
Cc: Eisemann, John D (APHIS)
Subject: Fw: Gonacon Immunocontraceptive Vaccine use on wildlife populations

Jack-Autumn Metzger from EPA called me today to ask how they should respond to an inquiry about GonaCon from Buffalo Field Campaign (see e-mail at the bottom of this string).

Autumn has worked with APHIS on general GonaCon registration issues under FIFRA for several years, but she's not familiar with the bison work because it hasn't involved any FIFRA pesticide registration issues. Obviously, as we all know, this will soon change because there will be some crossover next year between FIFRA and NEPA issues when GonaCon is used in the bison study.

We've talked as a group and with WS and VS about the need to obtain an Experimental Use Permit (EUP) for GonaCon bison work because it is already registered as a pesticide with EPA. We typically have preliminary meetings or discussions with EPA prior to submitting EUP's so they understand what's coming and are informed about the reasons for the EUP request. We haven't done that yet for the GonaCon bison study so the information about the bison study is new to EPA. Just as background information, I did tell Autumn that there is a study planned for next year and that as of now we expect to submit an EUP application later this year to cover the needed FIFRA approvals for the work.

Autumn didn't think EPA would be able to respond fully to the BFC questions because they have not been involved in the bison work. I told Autumn that VS might be a more logical group to respond fully to BFC's questions, so she forwarded the inquiry below to me to pass on.

Autumn asked me to let her know when APHIS responds to BFC so she knows the issue's been addressed.

Let me know if you want to talk about this further.

Thanks,

Stephanie
----- Forwarded by Stephanie H Stephens/MD/APHIS/USDA on 05/31/2011 01:41 PM -----

From: Metzger.Autumn@epamail.epa.gov
To: stephanie.H.Stephens@aphis.usda.gov
Date: 05/31/2011 01:07 PM
Subject: Fw: Gonacon Immunocontraceptive Vaccine use on wildlife populations

---

Hi Stephanie,

Nice catching up with you. See the email below. Thanks for forwarding this on to the PR team to answer his questions.

Stay warm!

Autumn Metzger

Biologist
U.S. Environmental Protection Agency
Insecticide-Rodenticide Branch
Registration Division (7505P)
1200 Pennsylvania Ave. NW
Washington, DC 20460

Tel: 703 305-5314
Fax: 703 308-5433
Email: metzger.autumn@epa.gov
----- Forwarded by Autumn Metzger/DC/USEPA/US on 05/31/2011 03:06 PM -----

From: Darrell Geist <z@wildrockies.org>
To: Autumn Metzger/DC/USEPA/US@EPA
Date: 05/30/2011 01:41 PM
Subject: Gonacon Immunocontraceptive Vaccine use on wildlife populations

Autumn Metzger, Registration Division
Office of Pesticide Programs, Environmental Protection Agency
1200 Pennsylvania Ave., NW.
Washington, DC 20460-0001
telephone number: (703) 305-5314
e-mail address: metzger.autumn@epa.gov

Dear Autumn Metzger,

I wanted to inquire if the Environmental Protection Agency has registered or requires registration for use of Gonacon Immunocontraceptive Vaccine on American bison.

The bison in question are part of the wildlife population under the jurisdiction of Yellowstone National Park, were captured this year in a trap that previously held quarantined bison from the same population, and were not released along with their cohorts this spring so that USDA APHIS could perform a 7-year study that administers Gonacon to the bison in captivity.

Ostensibly, Yellowstone National Park has issued a permit to USDA Animal and Plant Health Inspection Service to take bison from the wildlife population.

From May 26, 2011 Yellowstone National Park Press Release (online: http://www.nps.gov/yell/parknews/11047.htm)

"Fifty-three yearling through four-year-old bison remain in the Corwin Springs facility as a part of a USDA Animal and Plant Health Inspection Service initiated research project to determine whether brucellosis positive female bison can be prevented from shedding Brucella bacteria by
treating them with a contraceptive vaccine."

Any information you can provide on this matter would be appreciated.

--
Darrell Geist
Habitat Coordinator
Buffalo Field Campaign
PO Box 957

West Yellowstone MT 59758
phone: (406) 646-0070
fax: (406) 646-0071
email: z@wildrockies.org
http://www.buffalofieldcampaign.org/habitat.html

Are wild buffalo a threat to Montana's economy?
http://www.buffalofieldcampaign.org/faq/wildeconomy.html

Eisemann, John D - APHIS

From: Pauline Nol <pauline.nol@aphis.usda.gov>
Sent: Tuesday, March 08, 2011 8:36 AM
To: John D Eisemann
Cc: Jack C Rhyan
Subject: bison contraception protocol
Attachments: AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx

Hi John,
I think (other than a few nitty gritty things I need to fill in like references) that I've reached my limit on competence in filling out the protocol for the bison study.
Would you be able to take a look at this, especially NEPA and material appendices? Or send me in the right direction on who can help me with this?
Thanks!
Pauline

(See attached file: AD003-04 GonaConBisonStudy2011 QA 1858 draft 3.3.11.docx)

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA-APHIS-VS-Western Region
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Ph: (970) 266-6126
Cell:(970) 218-1418
Fax:(970) 266-6138
pauline.nol@aphis.usda.gov

1.1 United States Department of Agriculture

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

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) ☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☒ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix.**
Permits		
☐	☒	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. _____ _____ _____ _____ _____ _____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix.** Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☐	☒	Will this study be conducted under any regulatory standard? If yes please check: ☒ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☐	☒	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix.** Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☒	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement.**
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix.**

PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: May 1, 2011

End Date: October 1, 2015

Archive Date: October 1, 2016

Anticipated Project Outcome:
- ☒ Manuscript
- ☒ Report
- ☐ Other: _____

Materials to be archived to close this activity:
Raw data
Final Report

Description of Project and NWRC Activities and Participation:
See attached Research Plan

Comments:

Attachments: (e.g. Material Transfer Form, IACUC approval, etc.)	IACUC Protocol Approval Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: May 1, 2011
Proposed Experimental Termination Date: October1, 2015
Proposed Study Completion/Archive Date: October 1, 2016

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella* abortus, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* transmission in a bison herd.
2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison.

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites
2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison.
3. Evaluate *B. abortus* transmission to bison bulls during rut.

Hypotheses:

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

9. Methods/Procedures

A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 5 extra seronegative animals to allow for seroconversion immediately following capture and confinement) and 6 seronegative bulls captured in late winter/spring 2011 as part of the ongoing Interagency Bison Management Plan will be transported to the USDA/APHIS/VS bison facilities in Corwin Springs, Montana.

Routine procedures (collecting blood, fitting collars, etc.) will be done in the facility bison chute.

Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

Animals will be placed in the facility approximately one year prior to vaccination to allow exposed animals time to seroconvert prior to designation as seropositive or negative. If fewer than 45 bison are captured in Spring of 2011, they will be maintained in the facility until a sufficient cohort of animals are available.

The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations;

no cattle are present within a mile of the facilites. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

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

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

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

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

Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Daily observation for abortions, labor, and parturition events will be conducted. Serology for each of the cows, bulls, and calves will be monitored twice a year. In February each year, cows will be pregnancy tested and pregnant animals fitted with vaginal transmitters to alert investigators to abortion or calving events (Rhyan et al., 2009). Also, females will be fitted with collars carrying RFID sensors and/or cameras to record exposure of herd mates to aborted fetuses or parturition products. Following an abortion, the fetus will be left at the abortion site for 24 hours to monitor exposure of other animals. The fetus will then be collected, necropsied, and incinerated at the Montana Veterinary Diagnostic Laboratory (MVDL) in Bozeman, MT. All bison will be tested by serology in February and in summer following calving. At the end of the study, seropositive adult animals will be euthanized and necropsied with specimens collected for culture. Specimens for culture collected during the study will be maintained frozen at minus 70°C until the conclusion of the study and then shipped to the NVSL, Ames, IA for culture.Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques.

Offspring that remain or become seropositive for B. abortus after weaning will be euthanized and necropsied. Adults and offspring that remain negative for brucellosis on serology and culture and satisfy the bison quarantine requirements as published in the UM&R will be used for bison conservation. The exact process by which this will be done will be detailed in the spring of 2011 after the end of Montana's legislative session. It will likely utilize an independent organization such as the American Bison Society/Wildlife Conservation Society. Serologic tests will be conducted at the MVDL and/or National Veterinary Services Laboratories in Ames, IA throughout the study to ascertain the ongoing serologic status of each animal.

10. Experimental Design and Statistical Analyses

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

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

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental

	test samples

12. List of Records to be Maintained

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

13. Cost Estimate for Each Fiscal Year

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

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

[Standard text revise as needed] All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs.

16. Archiving

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

17. Protocol Amendments

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

18. References

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

Comment [pn1]: Still need to write these out

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

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

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

A. Animal Description

1) Animals:

Species, subspecies (if applicable): Bison (Bison bison)
Breed, strain and substrain (if applicable): NA
Total Number and Sex: 45 females, 6 males
Body weight range: 400-1000 kg
Age: 2 year to adult

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

1) Rationale for involving animals:

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

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate): The target number of animals in each group is 20, consisting of 10 seropositive animals and 10 seronegative animals. 5 extra seronegative animals will be collected as it is expected that a small percentage of seronegative animals captured will seroconvert during the first year before vaccination.
The study will determine whether there is a difference in the number of seroconversions in naïve animals exposed to *Brucella abortus*-infected animals who are allowed to breed naturally and those who are immunocontracepted with GonaCon. The number of animals to be assigned to the seronegative groups was determined using a power calculation in SAS (power for comparing 2 independent proportions). We consider power to be acceptable at a level of approximately 80%. We will be using a one-sided test and an alpha level of 0.05. The treatment will be deemed successful if the number of seroconversions in the seronegative group exposed to untreated seropositive animals exceeds that of seronegatives exposed to treated seropostives by 50% or more. A sample size of 10 per group was calculated to be sufficient in order to determine differences between treated and untreated groups under the above stated power and alpha constraints.

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

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

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

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

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

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

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

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

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

H. Quarantine
Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology.

I. Housing/maintenance
The animals will be housed and the study conducted in the double-fenced facilities utilized for the Bison Quarantine Feasibility Study located north of Gardiner, Montana. These facilities comprise 7 pastures totaling approximately 120 acres and holding corrals and working facilities.

J. Dietary contaminant exposure
NA

K. Disposition of animals
It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

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

L. Animal pain or distress

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

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

Name of Attending Veterinarian: _____

Date of Consultation: _____

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

☒ No

☐Yes If yes, continue with the following items.

a) Alternative procedures:

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

c) Surgery:

M. Euthanasia
It is not anticipated that any animals will require euthanasia until termination of the study. However, if an animal is fatally injured during routine handling, euthanasia will be administered either by captive bolt as animals will be chemically immobilized prior to euthanasia, or 88 mg/kg pentobarbital, as the situation requires. The carcasses of euthanized animals will be incinerated at the Montana Veterinary Diagnostic Lab or deposited in a secure landfill if one is available.

N. IACUC Approval

Date of IACUC Approval Letter:_____

O. Staff Qualifications

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

Comment [pn2]:

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does <u>not</u> 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 <u>target</u> 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 <u>non-target</u> species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

This study will have no impact on nontarget species

Effects on T&E species and eagles:

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

☒ No

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

Consultations:

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

☐ No

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

Comment [pn3]:

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

☐ No, permission not needed because:

Comment [pn4]:

☐ Yes

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

GnRH/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml
AdjuVac™ adjuvant	
Mycobacterium avium (Mycopar™ – *M. a. paratuberculosis*)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

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

D. Route of administration

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

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

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

Comment [pn5]: ??

TRCS tracking number(s):_____

G. Material verification

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

Comment [pn6]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:
ACP Consultation: _____ Date: _____

Eisemann, John D - APHIS

From: Pauline Nol <pauline.nol@aphis.usda.gov>
Sent: Friday, January 21, 2011 11:46 AM
To: Jack C Rhyan; John D Eisemann
Subject: Re: Bison Study protocol
Attachments: pic00428.gif

Yes please as I am not experienced in writing up GLP studies etc.

Jack C Rhyan---01/21/2011 09:41 AM MST---

From: Jack C Rhyan
To: John Eisemann
Cc: Pauline Nol
Date: 01/21/2011 09:41 AM MST
Subject: Re: Bison Study protocol

We'll definitely need your help!
Jack

John D Eisemann---01/21/2011 09:38:57 AM---OK. Pauline, I am more than willing to help seeing as how this will be a EPA regulated study. John Eisemann

John D Eisemann/CO/APHIS/USDA ToJack C Rhyan/CO/APHIS/USDA@USDA

01/21/2011 09:38 AM ccPauline Nol/CO/APHIS/USDA@USDA

Subject
Re: Bison Study protocol

OK. Pauline, I am more than willing to help seeing as how this will be a EPA regulated study.

John Eisemann
USDA APHIS Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80526

T: 970-266-6158
F: 970-266-6157

Jack C Rhyan---01/21/2011 09:21:14 AM---I figured. We'll get after it. Pauline is magic on these. She is out til next week. Jack

Jack C Rhyan/CO/APHIS/USDA ToJohn D Eisemann/CO/APHIS/USDA@USDA

Subject
Re: Bison Study protocol

I figured. We'll get after it. Pauline is magic on these. She is out til next week.
Jack

John D Eisemann---01/21/2011 08:31:06 AM---Jack, I am glad you sat in on QA's presentation of our new protocol template. Unfortunately the new template leads the Bison s

John D Eisemann/CO/APHIS/USDA

01/21/2011 08:30 AM

To Jack C Rhyan/CO/APHIS/USDA@USDA, Stephanie H Stephens/MD/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA

cc

Subject Bison Study protocol

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

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Eisemann, John D - APHIS

From: Jack C Rhyan <jack.c.rhyan@aphis.usda.gov>
Sent: Friday, January 21, 2011 9:41 AM
To: John D Eisemann
Cc: Pauline Nol
Subject: Re: Bison Study protocol
Attachments: pic04492.gif

We'll definitely need your help!
Jack

John D Eisemann---01/21/2011 09:38:57 AM---OK. Pauline, I am more than willing to help seeing as how this will be a EPA regulated study. John Eisemann

John D Eisemann/CO/APHIS/USDA To Jack C Rhyan/CO/APHIS/USDA@USDA
01/21/2011 09:38 AM cc Pauline Nol/CO/APHIS/USDA@USDA
Subject Re: Bison Study protocol

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Jack C Rhyan/CO/APHIS/USDA To John D Eisemann/CO/APHIS/USDA@USDA
01/21/2011 09:21 AM cc Pauline Nol/CO/APHIS/USDA@USDA
Subject Re: Bison Study protocol

I figured. We'll get after it. Pauline is magic on these. She is out til next week.
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John D Eisemann---01/21/2011 08:31:06 AM---Jack, I am glad you sat in on QA's presentation of our new protocol template. Unfortunately the new template leads the Bison s

000205

John D Eisemann/CO/APHIS/USDA

01/21/2011 08:30 AM

To Jack C Rhyan/CO/APHIS/USDA@USDA, Stephanie H Stephens/MD/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA

cc

Subject Bison Study protocol

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F: 970-266-6157

From: Jack C Rhyan <jack.c.rhyan@aphis.usda.gov>
Sent: Friday, January 21, 2011 9:21 AM
To: John D Eisemann
Cc: Pauline Nol
Subject: Re: Bison Study protocol
Attachments: pic26875.gif

I figured. We'll get after it. Pauline is magic on these. She is out til next week.
Jack

John D Eisemann---01/21/2011 08:31:06 AM---Jack, I am glad you sat in on QA's presentation of our new protocol template. Unfortunately the new template leads the Bison s

John D Eisemann/CO/APHIS/USDA

01/21/2011 08:30 AM

To Jack C Rhyan/CO/APHIS/USDA@USDA, Stephanie H Stephens/MD/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA

cc

Subject Bison Study protocol

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

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F: 970-266-6157

O'Hare, Jeanette R - APHIS

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

FYI – in case you have not seen yet. http://www.buffalofieldcampaign.org/media/press1011/pressreleases1011/053111.html

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

Family Planning on the Range: The Battle Over Bison Contraceptives

By Sarah Yager

Could contraceptives offer protection for the nation's last continuously wild herd of American bison?

At feeding time, residents of the Brogan Bison Facility cluster around a hay bale, blinking at flecks of alfalfa dust that swirl in the air and settle in their shaggy coats. The herd, chewing and lowing, mills in a holding pasture near Corwin Springs, Montana, surrounded by sweeping mountain views and a seven-strand wire fence. Blue-painted squeeze chutes are settled in the dirt nearby, bordered by a swath of prairie grass that stretches for a few miles until it meets the northern border of Yellowstone National Park. This, under a graying sky beginning to spit the first snowflakes of another long winter, is the unlikely center of a contentious debate over birth control.

The bison, gathered after drifting out of Yellowstone earlier this year, are potential subjects of a USDA study of GonaCon, a contraceptive vaccine for wildlife. Originally developed by the USDA as a non-lethal form of pest control, GonaCon works by lowering the concentration of sex hormones in the

bloodstream to weaken fertility and the urge to mate. The contraceptive was recently approved in Maryland and New Jersey for curbing the population of wild deer. Now researchers are hoping to use GonaCon to stop the spread of brucellosis, an infectious bacterial disease that causes pregnant ungulates to abort their calves.

The Greater Yellowstone Area is the last known reservoir of *Brucella abortus* bacteria, believed to have been introduced to the park's bison by domestic cattle at the beginning of the 20th century. Roughly half the bison population in Yellowstone tests positive for exposure to the disease, which is primarily transmitted by contaminated birthing materials deposited on grazing grounds. Brucellosis also poses a threat to neighboring cattle herds when infected animals wander over the park's invisible boundaries. Researchers from the USDA's Animal and Plant Health Inspection Service (APHIS) are interested in whether temporary sterilization with GonaCon can prevent the shedding of bacteria-riddled afterbirth and help block disease transmission.

The USDA has spent close to two billion dollars over nearly eight decades trying to stamp out the disease, which carries hulking environmental and financial consequences. Bison who leave the park to seek food at lower elevations are routinely rounded up and quarantined, and those found to have the disease are slaughtered. When brucellosis crops up on cattle ranches, herds must be quarantined and infected members butchered. Additionally, the bacteria can pass to humans through unpasteurized milk. Jack Rhyan, a veterinarian medical officer and wildlife pathologist with APHIS, and the study's principal investigator, said that the focus on brucellosis is driven in part by its implications for public safety. "Animal health is directly related to human health," he said.

But while the GonaCon study is still in the nascent stages, some conservationists are already voicing concerns. Stephany Seay, media coordinator of Buffalo Field Campaign, a group that advocates for protection of the Yellowstone herd, views the USDA study as an experiment in population control. "Brucellosis is being used as a tool to manipulate the movement of wild bison," she told me. According to Seay, GonaCon is a means of catering to ranchers who don't want to compete with bison for grassland. "This is a centuries-old range war," she continued.

Indeed, the interests of land-users have historically clashed with bison and their habitat. Once scattered over the Great Plains, the American bison population was demolished in the late 1800s by settlers hungry for meat, hides, and room for westward expansion. Numbers dwindled from an estimated 30 million to fewer than one thousand. By the turn of the century, Yellowstone held the nation's only remaining wild population of plains bison. Biologists have determined in recent years that the herd is one of the last to retain genetic purity, with no traces of interbreeding with cattle.

From Seay's perspective, the significance of the Yellowstone herd is reason to encourage tolerance over further tampering. She and the Buffalo Field Campaign have fought to expand range rights for bison. "The dispersal of wildlife lessens the prevalence of disease," she said. "By allowing bison to roam, you're thereby also reducing risks." Ranchers anxious about contagion, she suggested, could immunize their animals against brucellosis rather than meddle with neighboring wildlife.

But while bison remain in the essentially artificial environment of Yellowstone National Park, bounded by a patchwork of land and legal rights, some degree of management may be necessary--even beneficial --for the animals inside. Marty Zaluski, Montana's state veterinarian, pointed out that the goals of the USDA and bison advocates are, to some degree, in alignment. "It's a non-lethal method to reduce the

infection rate while slowing the population growth, and therefore reducing the number of animals that go to slaughter," he said. "I really don't comprehend why this is such a lightning rod for conservationists' concerns when you look at the alternatives."

Zaluski, who has also been a vocal advocate of immunization, sees GonaCon as a valuable addition to the disease-fighting quiver. He maintained that birth control could do more than buffer direct impacts of the disease in Yellowstone's herd. "Brucellosis is limiting the ability to take the bison from this area and restore them in other parts of the country," he said. GonaCon, with its potential to wipe out infection, could make the public more open to the concept of a free-roaming herd. "Ultimately, the entire nation loses by not being able to benefit from and enjoy Yellowstone bison."

USDA APHIS is in the process of conducting an environmental assessment to determine whether the proposed study should move ahead. The assessment is scheduled to wrap up by early January, and the results will be made available for public comment. If approved, work could begin this spring--around the time a new generation of bison calves tests their wobbly legs.

Image: Jim Parkin/Shutterstock.

This article available online at:

http://www.theatlantic.com/technology/archive/2011/11/family-planning-on-the-range-the-battle-over-bison-contraceptives/248851/

O'Hare, Jeanette R - APHIS

From: Nol, Pauline (APHIS)
Sent: Tuesday, August 16, 2011 1:50 PM
To: O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study

Thanks Jeanette!

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

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

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

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

Pauline,

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

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

Let me know if you need anything.

Jeanette

From: Nol, Pauline (APHIS)
Sent: Friday, June 03, 2011 3:24 PM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS);

O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study

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

Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

From: Eisemann, John D (APHIS)
Sent: Friday, June 03, 2011 10:46 AM
To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)
Subject: Meeting to discuss the Bison Study

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

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

1.1 United States Department of Agriculture

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

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) ☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☒ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix**.
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____National Park Service_____ _YELL-2011-SCI-5892_____ May 10, 2011____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix**. Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☐	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☐	☒	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☒	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement**.
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix**.

Comment [pn1]: ??

PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date:

Comment [pn2]:

Anticipated Project Outcome:
- ☒ Manuscript
- ☒ Report
- ☐ Other: _____

Materials to be archived to close this activity:
Raw data
Final Report

Description of Project and NWRC Activities and Participation:
See research plan

Comments:

Attachments: (e.g. Material Transfer Form, IACUC approval, etc.)	IACUC Protocol Approval Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2019
Proposed Study Completion/Archive Date:

Comment [pn3]:

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella* abortus, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

9. Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

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

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

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

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

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

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

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

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

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

10. Experimental Design and Statistical Analyses

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

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

Comment [pn4]:

13. Cost Estimate for Each Fiscal Year

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

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

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

16. Archiving

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

17. Protocol Amendments

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

18. References

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

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

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

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

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Azaperone- 0.02 mg/kg

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

13) Euthanasia

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

14) Staff Qualifications

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

N. IACUC Approval

Date of IACUC Approval Letter:__ACUC Protocol approved 5/17/2011_See attached____

Comment [pn5]: By Montana IACUC-Name?

O. Staff Qualifications

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

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

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

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

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

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? ☒ 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

Comment [pn6]:

Comment [pn7]:

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

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

D. Route of administration

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

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

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

Comment [pn8]: ??

TRCS tracking number(s):_____

G. Material verification

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

Comment [pn9]: ???

If materials are to be analyzed by the Analytical Chemistry Project complete the following:
ACP Consultation: _____ Date: _____

O'Hare, Jeanette R - APHIS

From: Nol, Pauline (APHIS)
Sent: Monday, June 06, 2011 1:45 PM
To: O'Hare, Jeanette R (APHIS); Eisemann, John D (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

I hijacked that information from the elk protocol. This can be changed however it needs to be changed.
Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

From: O'Hare, Jeanette R (APHIS)
Sent: Monday, June 06, 2011 1:44 PM
To: Eisemann, John D (APHIS); Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS)
Subject: RE: comments on bison protocol

Just a note to concur with John's comment in the protocol regarding the GonaCon formulation. What you have in the protocol right now is the currently registered product. Lowell has made several changes for a new formulation which have significant regulatory implications. We need to clarify this.

Jeanette

From: Eisemann, John D (APHIS)
Sent: Monday, June 06, 2011 11:03 AM
To: Nol, Pauline (APHIS); Rhyan, Jack C (APHIS)
Cc: Stephens, Stephanie H (APHIS); O'Hare, Jeanette R (APHIS)
Subject: comments on bison protocol

I am around all week if you want to discuss any of these comments.

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

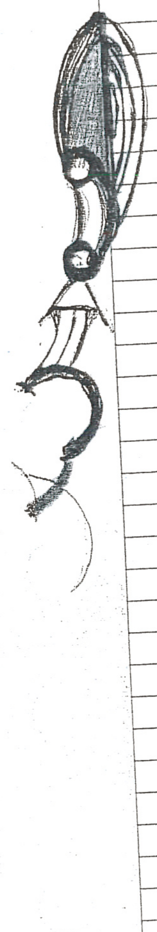
A fool sees not the same tree that a wise man sees.
—William Blake

Thursday
July 201

Day Planner
John Eisemann

Daily Notes

188th Day 177 Left Week 27



Call Cathy Bowie
- Campbell study GonaCon – GLP?

Call Pitt
-

Meeting w/ DO LM + KAF
- India – Tibet GonaCon Study
- where is the SenesTech licensing
  - if we go for a European patent APHIS will have to disclose to both interested parties
- We have no way to take $ unless it is through a CRADA
  - MUST BE TRUE RESEARCH
    - NOT OPERATIONAL USE
- SenesTech wants to do 800 dogs
  - what do they want to do?
  - SenesTech may be paying total cost
    - who is paying?

- if this study is truly research
  - do to we interest!
    - preventing disease
  - what is the study design?
    - will there be adequate IACUC oversight
  - what will we do w/ the data

⊗ Larry's Decision is to kill the European patent.

Conf Call w/ CB, KAF, JO, SS
- Bison in Yellowstone / GonaCon Study
- We will send EPA a letter asking if study is exempt from EUP
  - want answer in writing for file
- Sept is the deadline for EUP submission

Kathy Fagerstone Meeting Notes 000236 Hamilton

Conf Call re. Bison GonaCon Study 6/20/11
Stephanie Stephens, Jack Rhyan, Pauline Nol,
Jack Edmundson, Ann Nason, Tracy
Willard, — Re EA – Done by US.

MT, Mtg. 6/27/11
1. John E, back from AVPC –
David Dall – M-44 PAPP worked
really well for dogs
Registered in NZ for stoats – tunnel - paste
~~put on~~ squirted on stomach → lick off.
Gut length, paw pattern

Larry — SES leader call
APHIS reorganizing – Consolidate
business practices
Gene — 1. Tyler to FL
2. Will – Palmyra called — Bait took longer – Sea turtle died
3. 2 French vet students in Starkville

Gail — Highlights report went to WR and
was given out. We may need to
pay again.
Dan – 2 geese died after capture
Mark – Ken Wilson, Mike Yeary, Bill
Andelt, Dale Nolte. – CSU course.
Joyce — WR taken lead from FEMI program
First round of wish list made
* $2000 – Jay Kirkpatrick
* $2500 – Add to ARS agreement

Kathy Fagerstone Meeting Notes

Mtg. w/ Bison Study -- 7/7/11

Buffalo Fields enquired about legality of doing the bison study.

No NEPA for capture of bison - part of VS research - But NEPA needed

Stephanie:
To Do: Send letter to EPA saying we think ~~we don~~ no EUP is needed for bison study - pen study / injection -
Kim Nesbit = EPA person
Request response by August 1.

↑

Mtg. re. Cheryl Jope re. SOF --
Do you want me to
Email Lary - attend FLC mtg? - over
on travel -

Work plan for Jay Kirkpatrick 2000
Financial Plan

Spend this FY -
Can do the agreement for 1 year
Something
Early publicity for the conference
Pre-conference publicity.
They can bill us - if print in Oct -> can bill us now

G

Fagerstone, Kathleen A - APHIS

From: Jack C Rhyan <jack.c.rhyan@aphis.usda.gov>
Sent: Thursday, November 18, 2010 3:27 PM
To: John D Eisemann/CO/APHIS/USDA
Cc: Kathleen A Fagerstone; Lowell A Miller/CO/APHIS/USDA; jeff kemp
Subject: Draft Brief Proposal for bison contraception work
Attachments: ImmunocontBisonProject_11-18.doc

John,
Please let me know how much more detail we need for now.
Jack
(See attached file: ImmunocontBisonProject_11-18.doc)

Proposed Project:

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

Investigators:

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

USDA, APHIS, WS: Lowell Miller, Jeff Kemp

Background:

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

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

Major Objectives:

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

2. Evaluate the effect immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison

Minor Objectives:

1. Evaluate, by use of proximity collars, the risk and extent of exposure of herd members to parturition sites

2. Evaluate infection in calves born to and reared by *B. abortus* seropositive bison

3. Evaluate *B. abortus* transmission to bison bulls during rut.

Research Plan:

This general research plan will be followed. A total of 45 female bison (yearlings, two- and three-year-olds – animals born in 2010, 2009, and 2008, approximately 25 seronegative and 20 seropositive - 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. Seronegative animals will be separated from seropositives and monitored bi-monthly by serology until August and semi-annually thereafter. Bulls will be maintained separately and monitored by serology. The animals will be housed and the study conducted in the double-fenced facilities utilized for the bison quarantine feasibility study located north of Gardiner, Montana. This location is within the Greater Yellowstone Area where brucellosis is endemic in bison and elk populations. 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 sentinel bison will be fitted with proximity collars programmed to record proximity to one another and to transmitters on vaginal implants. Following the first exposure to the bulls in 2012, three calving seasons will be observed (2013, 2014, and 2015). Bulls will be separated from the cows after breeding season, from December til July. During the three abortion/calving seasons (from February til August), reproductive outcomes for each of the cows will be monitored. Serology for each of the cows, bulls and calves will be monitored twice a year. In February each year, animals will be pregnancy tested and pregnant animals fitted with vaginal transmitters. Transmitters will alert investigators to abortion

or calving events and record exposure of sentinel animals. Animals will be tested by serology in February and in summer following calving. At the end of the study, all adult animals will be euthanized and necropsied with specimens collected for culture. Ova and semen will be collected and frozen for genetic conservation utilizing embryo transfer techniques. Offspring from seropositive cows will be euthanized and specimens collected for culture when calves are between 8 and 12 months of age. Caracsses will be donated to the Montana Food Bank. Offspring from seronegative cows will be ovarectomized or neutered and the animals provided to Tribes or donated to the Montana Food Bank when calves are between 8 and 12 months of age.

Time line:
Winter/spring 2011 – Transport bison to Corwin Springs facility and begin serologic testing. Separate into groups of seropositive and seronegative animals, keep bulls separate.
Spring 2012 – Place groups in pastures for study; in July, introduce bulls.
Winter/Spring 2013-2015 – monitor herds for calves, abortions, and seroconversions. Separate bulls from cows from December til July each year. When calves are 8 to 12 months of age, donate to MT Food Bank or neuter and donate to Tribes.
Summer 2015 – Euthanize, necropsy and culture study animals, collect ova and semen for genetic conservation.

Expected outcomes:

1. The effectiveness of the immunocontraceptive vaccine GonaCon™ in reducing transmission of *B. abortus* in bison herds will be determined.

2. The effect of prolonged anestrus produced by GonaCon™ on the survival of *B. abortus* in infected bison will be determined.

3. The risk and extent of exposure of bison herd members to *B. abortus* at parturition sites (in a captive setting) will be determined.

4. The nature of infection (transient or ongoing) in calves due to suckling of seropositive cows will be determined.

5. The risk of venereal transmission of *B. abortus* to seronegative bull bison will be examined.

Fagerstone, Kathleen A - APHIS

From: Kathleen A Fagerstone <kathleen.a.fagerstone@aphis.usda.gov>
Sent: Friday, December 03, 2010 2:00 PM
To: Jack C Rhyan
Cc: John D Eisemann/CO/APHIS/USDA; Lowell A Miller/CO/APHIS/USDA; Matt McCollum; Pauline Nol
Subject: Re: bison contraception project
Attachments: pic23442.gif; pic29866.gif

Lowell, Did you think that John WAS available on the 14-16th?
Regardless, the 13th works for me.
Kathy

Jack C Rhyan---12/03/2010 01:25:33 PM---Soooo.o.o. John is not available on the 14th - 16th but this time is okay with Lowell. How about the 13th at 10 in the mornin

Jack C Rhyan/CO/APHIS/USDA
12/03/2010 01:29 PM

To John D Eisemann/CO/APHIS/USDA@USDA

cc Kathleen A Fagerstone/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

Soooo.o.o. John is not available on the 14th - 16th but this time is okay with Lowell. How about the 13th at 10 in the morning. Would that work for anyone?
Jack

John D Eisemann---12/02/2010 11:40:41 AM---That works for me. I think the only time I am unavailable is Dec. 14-16. John Eisemann

John D Eisemann/CO/APHIS/USDA
12/02/2010 11:40 AM

To Kathleen A Fagerstone/CO/APHIS/USDA@USDA

cc Jack C Rhyan/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

That works for me. I think the only time I am unavailable is Dec. 14-16.

John Eisemann
USDA APHIS Wildlife Services
National Wildlife Research Center

4101 LaPorte Avenue
Fort Collins, CO 80526

T: 970-266-6158
F: 970-266-6157

Kathleen A Fagerstone---12/02/2010 11:39:13 AM---me too.

Kathleen A Fagerstone/CO/APHIS/USDA

12/02/2010 11:35 AM

To Jack C Rhyan/CO/APHIS/USDA@USDA

cc John D Eisemann/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject Re: bison contraception project

me too.

Jack C Rhyan---12/02/2010 10:46:27 AM---Kathy et al, We should meet soon to strategize on the bison project. I'm around mostly til Christmas.

Jack C Rhyan/CO/APHIS/USDA

12/02/2010 10:50 AM

To Kathleen A Fagerstone/CO/APHIS/USDA@USDA, John D Eisemann/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA

cc

Subject bison contraception project

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

Fagerstone, Kathleen A - APHIS

From: Jack C Rhyan <jack.c.rhyan@aphis.usda.gov>
Sent: Friday, December 03, 2010 4:56 PM
To: Matt McCollum
Cc: John D Eisemann/CO/APHIS/USDA; Kathleen A Fagerstone; Lowell A Miller/CO/APHIS/USDA; Pauline Nol
Subject: Re: bison contraception project
Attachments: pic29674.gif; pic29715.gif; pic30943.gif

10 am on the 13th sounds like the winner if Lowell can make it.
Jack

Matt McCollum---12/03/2010 03:36:21 PM---That works for me.

Matt McCollum/CO/APHIS/USDA

12/03/2010 03:32 PM

To John D Eisemann/CO/APHIS/USDA@USDA

cc Jack C Rhyan/CO/APHIS/USDA@USDA, Kathleen A Fasogerstone/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

That works for me.

John D Eisemann/CO/APHIS/USDA

John D Eisemann/CO/APHIS/USDA

12/03/2010 02:12 PM

To Kathleen A Fagerstone/CO/APHIS/USDA@USDA

cc Jack C Rhyan/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

I was wondering that myself. I can meet at 10 am on the 13th.

John Eisemann
USDA APHIS Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80526

T: 970-266-6158
F: 970-266-6157

Kathleen A Fagerstone---12/03/2010 02:06:07 PM---Lowell, Did you think that John WAS available on the 14-16th? Regardless, the 13th works for me.

Kathleen A Fagerstone/CO/APHIS/USDA

12/03/2010 02:01 PM

To Jack C Rhyan/CO/APHIS/USDA@USDA

cc John D Eisemann/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

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Regardless, the 13th works for me.
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Jack C Rhyan/CO/APHIS/USDA

12/03/2010 01:29 PM

To John D Eisemann/CO/APHIS/USDA@USDA

cc Kathleen A Fagerstone/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

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John D Eisemann/CO/APHIS/USDA

12/02/2010 11:40 AM

To Kathleen A Fagerstone/CO/APHIS/USDA@USDA

cc Jack C Rhyan/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject
Re: bison contraception project

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John Eisemann
USDA APHIS Wildlife Services
National Wildlife Research Center
4101 LaPorte Avenue
Fort Collins, CO 80526

T: 970-266-6158
F: 970-266-6157

Kathleen A Fagerstone---12/02/2010 11:39:13 AM---me too.

Kathleen A Fagerstone/CO/APHIS/USDA

12/02/2010 11:35 AM

To Jack C Rhyan/CO/APHIS/USDA@USDA

cc John D Eisemann/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA

Subject Re: bison contraception project

me too.

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Jack C Rhyan/CO/APHIS/USDA

12/02/2010 10:50 AM

To Kathleen A Fagerstone/CO/APHIS/USDA@USDA, John D Eisemann/CO/APHIS/USDA@USDA, Pauline Nol/CO/APHIS/USDA@USDA, Matt McCollum/CO/APHIS/USDA@USDA, Lowell A Miller/CO/APHIS/USDA@USDA

cc

Subject bison contraception project

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

Fagerstone, Kathleen A - APHIS

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, June 21, 2011 11:24 AM
To: Fagerstone, Kathleen A (APHIS)
Cc: Nol, Pauline (APHIS); Miller, Lowell A (APHIS)
Subject: RE: GonaCon Conference Call

Sure, I put our names on the Products conf room (Mt Princeton) See you all at 1:30 today. Kathy, please let John know if you think he needs to be there.

Jack

From: Fagerstone, Kathleen A (APHIS)
Sent: Tuesday, June 21, 2011 10:55 AM
To: Rhyan, Jack C (APHIS)
Subject: RE: GonaCon Conference Call

Jack—Do we want to all call from one phone?
Kathy

From: Rhyan, Jack C (APHIS)
Sent: Tuesday, June 21, 2011 10:08 AM
To: Nol, Pauline (APHIS); Fagerstone, Kathleen A (APHIS); Miller, Lowell A (APHIS)
Subject: FW: GonaCon Conference Call

FYI

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

Hi Everyone-

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

Date: Tuesday, June 21, 2011
Time: 3:30 ET (1:30 MT)
Phone: 888-858-2144
Code: 9514972

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

Thanks,

Stephanie

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

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

Hi, Jack. We pulled the Bison Team together the other day to begin work in earnest on the GonaCon EA. The first thing we did was go through the protocol with a fine-tooted comb to be sure we understood exactly what we are planning to do. Based on some things we have seen from BFC we suspect that they will be all over the study and watching like a hawk. As I understand it, the propocol you sent us is the final one that has been approved by NPS and a permit has been issued based on it. (In other words, APHIS shouldn't change anything in it because it would be a major paperwork hassle.) With that as background, we do have a few comments/questions about the protocol:

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

There are additional small points we would want to just talk with you about to get them straight in our minds or to ask your advice as to how to best present them in an EA. Can we organize a conference call with you to talk some of these things out? Since I am getting ready to retire, I'll be phasing out of the bison business (one of my regrets at retiring) and

Stephanie Stephens will be taking my place. Since she (and Vicki) will be leading the NEPA effort, she will be getting in contact with you to set up the conference call, but we wanted you to have at least a partial list of the things we have been thinking about.

Jack E

Fagerstone, Kathleen A - APHIS

From: Nol, Pauline (APHIS)
Sent: Friday, June 03, 2011 3:24 PM
To: Eisemann, John D (APHIS); Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); O'Hare, Jeanette R (APHIS)
Subject: RE: Meeting to discuss the Bison Study
Attachments: AD003-04 GonaConBisonStudy2011 QA 1858 draft_6.3.11.docx

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

Pauline

****
****

Pauline Nol, DVM, MS, PhD
Wildlife Livestock Disease Investigations Team
USDA APHIS VS WRO
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone: (970) 266-6126
Mobile: (970) 218-1418

From: Eisemann, John D (APHIS)
Sent: Friday, June 03, 2011 10:46 AM
To: Fagerstone, Kathleen A (APHIS); Rhyan, Jack C (APHIS); Miller, Lowell A (APHIS); Stephens, Stephanie H (APHIS); Nol, Pauline (APHIS)
Subject: Meeting to discuss the Bison Study

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

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

1.1 United States Department of Agriculture

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

PROTOCOL COVER PAGE

Study Title:	
NWRC Study Director:	
Approved NWRC Project:	

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ 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
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the 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)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the 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.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 2 (Regulatory Considerations) ☐ Part 4 (full NWRC Study Protocol) ☐ Complete and attach any appendices required 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".

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: _____

Position (check one):

☐ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☐ Res. Scientist ☐ Proj. Leader

☐ Research Scientist

☒ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	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. ☐ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☒	☐	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix**.
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. _____National Park Service_____ _YELL-2011-SCI-5892_____ May 10, 2011____ _____ _____ _____ Permit(s) description Number Date
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☐	☒	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix**. Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☐	☒	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☐	☒	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☐	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐Other:_____
☐	☒	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☐	☒	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☒	☐	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement**.
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix**.

Comment [pn1]: ??

PART THREE: DESCRIPTION OF ACTIVITIES

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

Collaboration:	Name	Address or Organization	Role in Project
	Jack Rhyan	USDA, APHIS, VS	Principle Investigator
	Rebecca Frey, Pauline Nol, Ryan Clarke, Matt McCollum, Luke Wagner	USDA, APHIS, VS	Investigators
	Lowell Miller, Kathy Fagerstone	USDA, APHIS, WS, NWRC	Investigators

Start Date: June 1, 2011

End Date: October 1, 2019

Archive Date:

Comment [pn2]:

Anticipated Project Outcome:
- ☒ Manuscript
- ☒ Report
- ☐ Other: _____

Materials to be archived to close this activity:
Raw data
Final Report

Description of Project and NWRC Activities and Participation:
See research plan

Comments:

Attachments: (e.g. Material Transfer Form, IACUC approval, etc.)	IACUC Protocol Approval Test, Control and Reference Material/Devices Formulation and Use Appendix.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Jack Rhyan	USDA, APHIS, VS	Principle Investigator
Other Investigators, Collaborators, Cooperators, and Consultants		
Rebecca Frey	USDA, APHIS, VS	Investigator
Pauline Nol	USDA, APHIS, VS	Investigator
Matt McCollum	USDA, APHIS, VS	Investigator
Ryan Clarke	USDA, APHIS, VS	Investigator
Luke Wagner	USDA, APHIS, VS	Investigator
Lowell Miller	USDA, APHIS, WS	Investigator
Kathy Fagerstone	USDA, APHIS, WS	Investigator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine), GLP (Good Laboratory Practices) compliance, and preparation of final report on GonaCon™ for submission to the US Environmental Protection Agency (EPA)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2019
Proposed Study Completion/Archive Date:

Comment [pn3]:

5. Background and Justification

Bovine brucellosis, a zoonotic bacterial disease caused by *Brucella* abortus, is transmitted among animals, including cattle, bison (*Bison bison*) and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Additionally, the organism is shed in the milk from infected dams and can be transmitted to cows through suckling. Following infection, females often abort. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may result in shedding of the organism. The occurrence of venereal transmission of brucellosis in bison in unknown; however, based on a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is not considered likely to be a significant route of transmission. Therefore, transmission of disease in cattle, bison and elk, is primarily dependent on the occurrence of pregnancy and abortion or calving of infected animals.
GonaCon™, an immunocontraceptive vaccine approved for use in wild white-tailed deer, has been shown to produce temporary infertility in female bison. In limited studies, infertility has lasted 3 years or longer following a single injection of 1800 µg or 3000 µg. Its use has been proposed as a nonlethal method of decreasing the prevalence of brucellosis in bison by preventing pregnancy and abortion or normal parturition and thereby preventing transmission of *B. abortus*.

6. Related Protocols

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

7. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

8. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

9. Methods/Procedures

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

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

Seronegative animals will be separated from seropositives and monitored every month by serology until August and three times a year thereafter. Bulls will be maintained separately and monitored by serology.

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

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

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

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

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

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

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

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

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

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

10. Experimental Design and Statistical Analyses

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

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

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AD 001.01	Standard Operating Procedures
AD 002.00	Quality Assurance Unit
AD 012.02	Test, Control, & Reference Substance Chain of Custody
AD 011.02	Data Recording and Error Correction
AD 003.03	Research Protocols
AD 010.01	Standard Format for Data Submissions to EPA
AD 004.01	Archiving Studies
BT 004.01	injection procedure for immunizing animals with immunocontraceptive vaccines
HS004-00	Personal protective equipment
BT 001.00	ELISA procedure for assessing immune responses
BT 016.02	Manufacture of GonaCon Immunocontraceptive Vaccine
HS013-02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

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

Comment [pn4]:

13. Cost Estimate for Each Fiscal Year

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

14. Human Health and Safety

HS004-00	Personal protective equipment

15. Staff Qualifications

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

16. Archiving

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

17. Protocol Amendments

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

18. References

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

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

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

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

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☒ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Azaperone- 0.02 mg/kg

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

13) Euthanasia

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

14) Staff Qualifications

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

N. IACUC Approval

Date of IACUC Approval Letter:__ACUC Protocol approved 5/17/2011_See attached____

Comment [pn5]: By Montana IACUC-Name?

O. Staff Qualifications

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

NEPA and ESA Appendix

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

This study qualifies for a Categorical Exclusion because:

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

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

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

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

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

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

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

This study will have no impact on nontarget species

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? ☒ 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

Comment [pn6]:

Comment [pn7]:

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

A. Describe the test material/devices

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

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

B. Describe any control or reference materials/devices

No control or reference materials will be used

C. Carriers, mixtures and material preparation

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

GnRH/KLH Conjugate (1000 µg)	
Mammalian Gonadotropin Releasing Hormone (GnRH)	0.300 mg
Concholepas concholepas hemocyanin (Blue))	0.760 mg
Phosphate buffered saline (tablets)	26.01 mg
Sucrose	5.46 mg
Sterile, ultrapure water	0.48 ml
AdjuVac™ adjuvant	
Mycobacterium avium (Mycopar™ – *M. a. paratuberculosis*)	0.170 mg
Light mineral oil	0.45 ml
Mannide monooleate	0.05 ml

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

D. Route of administration

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

E. Dosage

GonaCon™ will be administered via two intramuscular injections of 1500 ug in 1.5 ml volume. Booster injections of two intramuscular injections of 1500 ug in 1.5 ml volume will be administered one year later to ensure sterility of the animals.

F. Test, control, and reference substance accountability

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

Comment [pn8]: ??

TRCS tracking number(s):_____

G. Material verification

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

Comment [pn9]: ???

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

ACP Consultation: _____ Date: _____

Fagerstone, Kathleen A - APHIS

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

I assume you have seen this one.

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

FYI – in case you have not seen yet. http://www.buffalofieldcampaign.org/media/press1011/pressreleases1011/053111.html

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

Freeman, Nancy - APHIS

From: Miller, Lowell A - APHIS
Sent: Wednesday, April 18, 2012 2:45 PM
To: Freeman, Nancy - APHIS
Subject: FW: copy of IACUC for bison GonaCon study
Attachments: ACUCBisonGonaConStudyfinal.pdf; ACUC Comm signaturesGonaConBisonStudy.pdf

I found this e-mail

Lowell A. Miller Ph.D.
National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521
Phone (970) 266- 6163
Fax (970) 266-6157
e-mail: Lowell.A.Miller@aphis.usda.gov

From: Rhyan, Jack C (APHIS)
Sent: Thursday, June 02, 2011 4:14 PM
To: Miller, Lowell A (APHIS); Fagerstone, Kathleen A (APHIS)
Subject: copy of IACUC for bison GonaCon study

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

REGULATORY CONSIDERATIONS

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

DESCRIPTION OF ACTIVITIES

Nature of the Collaboration:

- ☐ *Advisory Committee participation*
- ☒ *Manuscript/review article collaboration*
- ☐ *Training program requiring the use of animals*
- ☒ *Data analysis, interpretation and reporting*
- ☒ *Other: ___Live animal work_____*

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

Start Date: June 1, 2011

End Date: October 1, 2017

STUDY PROTOCOL

1. Key Personnel

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

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

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Pre-study quarantine facility
USDA/APHIS/VS Bison Quarantine Feasibility Study Location	772 Highway 89, Corwin Springs, Gardiner, MT 59030	Testing site/housing facility
Montana Veterinary Diagnostic Laboratory	South 19th and Lincoln, Bozeman, MT 59718	Fetus sample collection and incineration
National Veterinary Services Laboratory	1920 Dayton Avenue, Ames, IA 50010	Serologic testing, culture, and histopathologic analysis
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Source of test material (GonaCon™ vaccine)
National Wildlife Research Center	4101 LaPorte Avenue, Fort Collins, CO, 80521	Serologic testing

3. Sponsor

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

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: October1, 2019

5. Background and Justification

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

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

6. Assurance of Non-Duplication of Studies

Studies using GonaCon™ as an immunocontraceptive have been conducted in elk, white-tailed deer, bison, and domestic dogs (Miller LA, Rhyan JC, and Drew, M, 2004). However, the use of GonaCon™ as an effective means of decreasing the prevalence of *Brucella abortus* in bison has not been studied to date.

The following databases were searched:
PubMed on 2/14/11: key word combinations were GnRH and bison; GonaCon and bison; contraceptive and bison

7. Objective/Hypotheses

Major Objectives:

1. Evaluate the effect of infertility produced by immunocontraception of *B. abortus*-seropositive female bison on *B. abortus* shedding in a bison herd.
2. Evaluate the effects immunocontraceptive vaccine-induced prolonged anestrous has on *B. abortus* colonization in naturally-infected female bison
3. Determine the nature of infection (transient or ongoing) in calves due to birth to and suckling of seropositive cows; determine pregnancy outcomes in calves born to seropositive dams.

Hypotheses:

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

8. Methods/Procedures

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

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

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

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

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

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

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

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

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

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

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

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

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

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

IA.

10. Experimental Design and Statistical Analyses

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

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

11. Animal Care and Use Information

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

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

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

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

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

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

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

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

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

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

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

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

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

Reversal for BAM:
Atipamezole 0.0375-0.03 mg/kg IM
Naltrexone 0.05-0.125mg/kg IM
Tolazoline 1 mg/kg IM

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

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

11) Animal pain or distress

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

Name of Attending Veterinarian: ___Patrick Ryan Clarke_____

Date of Consultation: _____13 May 2011_____

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

☒ No

☐Yes If yes, continue with the following items.

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

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

12. Staff Qualifications

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

13. References

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

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

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

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

SIGNATURE PAGE

Study Director Jack C. Ryan Date 5/16/2011

Concur

IACUC Chair _____ Date _____

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PART ONE: SIGNATURE PAGE

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

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

IACUC committee member [signature] 5/24/11

IACUC committee member _____ _____

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Attn: Jerry Wiscomb

PART ONE: SIGNATURE PAGE

Study Director: _____ Date: 5/16/11

Concur:
IACUC Chair _____ Date 5/16/11

IACUC committee member _____ 5/25/11
Jerry Wiscomb

IACUC committee member _____ _____

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