



ENVIRONMENTAL ASSESSMENT



Photo courtesy of Dean Biggins, USGS

FIELD STUDIES TO ASSESS THE SAFETY OF SYLVATIC PLAGUE VACCINE IN PRAIRIE DOGS AND NON-TARGET ANIMALS

April 2012

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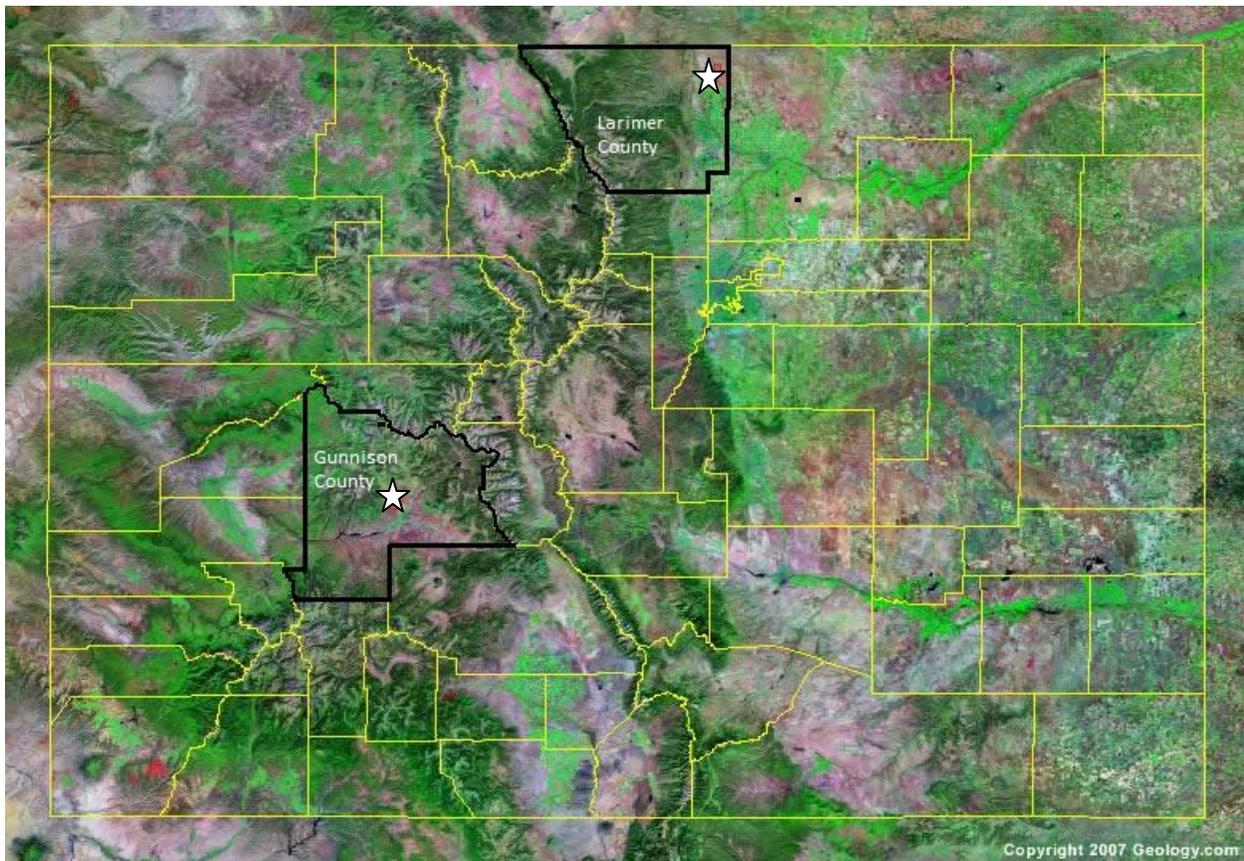
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| ACUC | Animal Care and Use Committee |
| BLM | Bureau of Land Management |
| BSL | Biosafety level |
| CPW | Colorado Parks and Wildlife |
| DNA | Deoxyribonucleic acid |
| EA | Environmental assessment |
| EIS | Environmental impact statement |
| FDA | Food and Drug Administration |
| LD50 | Lethal dose 50% |
| NEPA | National Environmental Policy Act |
| NWHC | National Wildlife Health Center |
| NWR | National wildlife refuge |
| PIT | Passive integrated transponder |
| RCN | Raccoon poxvirus |
| SCID | Severe combined immune deficient |
| SPV | Sylvatic plague vaccine |
| SWA | State wildlife area |
| <i>tk</i> | Thymidine kinase |
| USFWS | United States Fish and Wildlife Service |
| USGS | United States Geological Survey |

ENVIRONMENTAL ASSESSMENT OF FIELD STUDIES TO ASSESS THE SAFETY OF SYLVATIC PLAGUE VACCINE IN PRAIRIE DOGS AND NON-TARGET ANIMALS

1. INTRODUCTION

The U.S. Geological Survey (USGS) National Wildlife Health Center (Madison, WI) is proposing to conduct a field study to assess the safety of an oral sylvatic plague vaccine (designated as SPV) designed to prevent plague outbreaks in prairie dogs. The experimental vaccine uses raccoon poxvirus to carry genes of *Yersinia pestis*, the causative agent of plague. Inoculation with SPV via ingestion is capable of conferring protective immunity against plague to vaccinated prairie dogs. Prevention of plague in prairie dogs is a vital concern for ongoing conservation efforts and recovery programs for both prairie dogs and endangered black-footed ferrets which depend on prairie dogs for food and shelter. The field studies will be performed in restricted sites (See Figure 1) in Colorado within colonies of prairie dogs (*Cynomys* spp.) to assess the safety of SPV in wild prairie dogs and non-target animals, particularly rodents.

Figure 1. Aerial Photo Showing Study Areas in Larimer and Gunnison Counties



2. HISTORY

Plague, caused by the bacterium *Yersinia pestis*, is a disease of wild rodents that can afflict humans as well as other mammals and is well-known for its devastating effects on human and animal populations throughout history. Plague was introduced into North America around 1900 (Perry and Fetherston, 1997; Gage and Kosoy, 2005). Since then, numerous North American animal species have become infected with *Y. pestis*, with some species, such as prairie dogs (*Cynomys* spp.) and black-footed ferrets (*Mustela nigripes*), more severely affected than others. All prairie dog species have shown high susceptibility to plague, including the threatened Utah prairie dog and the candidate Gunnison's prairie dog, and can suffer >90% mortality during outbreaks (Lechleitner et al., 1968; Rayor, 1985; Ubico et al., 1988; Cully et al., 1997; Pauli et al., 2006). In prairie dogs, plague often results in reduced colony sizes, increased variation in local population sizes, and increased distances between colonies, making individual colonies more vulnerable to extinction (Miller and Ceballos, 1994; Antolin et al., 2002; Proctor et al., 2006). Black-footed ferrets are also highly susceptible to plague, contracting the disease by ingestion of infected prey or via bites from infected fleas (Williams et al., 1994; Rocke et al., 2004a, 2006, 2008a; Godbey et al., 2006). Prairie dogs are the primary prey of ferrets and prairie dog population declines that result from plague outbreaks are devastating for ferret populations as they rely almost exclusively on prairie dogs for their survival. Plague is considered to be prevalent throughout the western states and has been identified as a major threat to the conservation and/or recovery of both prairie dogs and black-footed ferrets.

For years, prairie dogs were considered to be epizootic hosts of plague, with outbreaks occurring through transmission via unknown enzootic reservoirs (Gage and Kosoy, 2005). However, recent evidence suggests that plague may also exist in an enzootic state among some prairie dog populations (Hanson et al., 2007; Biggins et al., 2010; Matchett et al., 2010) with epizootics triggered periodically, and unpredictably, by events not fully understood. Outbreaks of plague continue to occur in grassland communities throughout the western United States and threaten the recovery of the endangered black-footed ferret by decimating populations of prairie dogs, their primary prey. Management of plague in prairie dogs is a critical step in continuing progress in the recovery of the endangered black-footed ferret and the conservation of remnant grassland communities. In addition, management of plague in prairie dogs may have public health benefits. Approximately 14% of all human cases of plague in the U.S. since 1965 have been attributed to contact with black-tailed and Gunnison's prairie dogs and their fleas (Seery et al., 2003). Following the example of the "One Health" movement <http://www.onehealthinitiative.com/>, collaboration from agencies involved in wildlife health, environmental protection, and public health will be necessary to provide the resources and scientific inquiries needed to tackle the complex problem of plague.

3. PURPOSE AND NEED FOR THE ACTION

The purpose of the proposed action is to assess the safety of the SPV in wild prairie dogs and non-target animals after distribution in the field. Non-target animals include species other than prairie dogs that may ingest the vaccine-laden baits, such as other rodents, carnivores, deer, and domestic animals. The need for these actions is explained below.

In the past 100 years, plague has contributed to population declines of prairie dogs, near extinction of black-footed ferrets, and has caused human illness and fatalities in regions where

prairie dogs reside. Currently, the primary method for combating plague is insecticidal dusting of prairie dog burrows to control fleas, the vector of plague. However, dusting is labor intensive, time-consuming, short-lived, and impractical in some settings, especially large prairie dog complexes and in certain habitats. Also, weather can interfere with timely application of insecticidal dust.

An oral SPV, developed and tested jointly by the USGS, National Wildlife Health Center (NWHC) and University of Wisconsin (Madison, WI), is intended as a pre-emptive method for controlling plague in prairie dogs, without the disadvantages and potential collateral environmental effects of dusting. The use of SPV to prevent plague outbreaks in targeted prairie dog complexes, particularly where black-footed ferrets have been released or where prairie dog species conservation is a goal, could have both economic and environmental benefits. A plague vaccination program could enhance prairie dog and ferret recovery efforts, reduce pesticide use on public lands, allow managers to balance land use needs (agriculture and development) with conservation efforts on other sites, and protect human health in urban, recreational, military, and tribal areas with prairie dogs.

The SPV is a genetically modified viral vaccine, using attenuated raccoon pox virus as a vector for orally delivering critical plague antigens to target animals. Raccoon pox virus has been shown to be highly safe in numerous animals (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a; Mencher et al., 2004; Rocke et al., 2004a, 2006, 2008ab, 2010ab, unpublished), including black-footed ferrets, prairie dogs, dogs, cats, sheep, mice, etc. Initial laboratory trials demonstrate that consumption of baits containing this vaccine effectively protects prairie dogs from major encounters with virulent *Yersinia pestis* (Rocke, et al. 2010a). An effective bait that is highly palatable to prairie dogs and stable for the vaccine has been selected for field delivery. Bait ingredients are food grade, FDA approved and used within regulatory limits and contain potassium sorbate (<2% concentration) as a preservative and Rhodamine B (<0.5% concentration) as a biomarker. Biomarkers are regularly incorporated into baits to evaluate the success of bait distribution studies or to identify animals that have consumed vaccine-laden bait. Rhodamine B is an analytical dye that has been widely used as a marker and tracer in animal studies that marks hair, feces, or blood (Evans and Griffith, 1973; Johns and Pans, 1981; Lindsey, 1983; Fisher, et al., 1999). In preliminary field trials using baits without vaccine in multiple prairie dog species, bait acceptance rates of >90% have been observed (Rocke, unpublished data; Tripp, unpublished data). Experimental field trials are needed to confirm the consumption and safety of SPV in wild prairie dogs and non-target animals.

The continual presence of plague and the devastating effects of epizootics on all three prairie dog species (Gunnison's, black-tailed, and white-tailed) native to Colorado are well documented (Tripp et al., 2009; Griffin et al., 2010; Tripp et al., unpublished). Plague-related declines in abundance have negative effects, both on the long term persistence of prairie dog populations and on the multiple wildlife species that depend on prairie dogs for habitat and/or prey, including the endangered black-footed ferret (Antolin et al., 2002; Seglund and Schnurr, 2009). Confirmation of the safety of SPV in wild prairie dogs under field conditions will enable subsequent studies assessing the efficacy of SPV under field conditions with the ultimate goal of

using SPV as a management tool for the conservation of prairie dogs, the recovery of black-footed ferrets, and protection of public health.

3.1 Decision to be made

Based on the scope of this EA, the following questions must be answered:

- Should USGS undertake field trials in Larimer, Gunnison, and Moffat Counties, CO to determine the safety of SPV in prairie dogs and non-target animals?
- If not, should USGS implement another alternative?
- Would implementing the proposed action or an alternative action have significant adverse impacts on the quality of the human environment requiring the preparation of an EIS?
- When to conduct the trial?

3.2 Scoping and issues

3.2.1 Actions analyzed

This EA evaluates the environmental effects of distribution of SPV to assess the safety of the vaccine in wild prairie dogs and non-target animals.

3.2.2 Site specificity

The analysis of alternatives is limited to the study sites in Colorado and the associated species and habitats, as described in Section 5.

3.3 Summary of public involvement

In open houses held in Wellington, Colorado, on February 15, 2012, and in Gunnison, Colorado, on February 16, 2012, the USGS and Colorado Parks and Wildlife (CPW) consulted with members of the public regarding the proposed field studies to communicate to interested parties the analysis of potential environmental impacts. Publication of these meetings appeared in local businesses and colleges, community newspapers, and on community bulletin boards from January 25, 2012, through February 16, 2012, announcing development of an Environmental Assessment. Landowners with property adjacent to the study areas were mailed invitations to the meetings on February 1, 2012. Comments were recorded but no significant issues or concerns were raised during the open houses. See Appendix A for details on the open houses, including announcements and lists of attendees. Information available to the public is found in Appendix D and at the USGS NWHC website http://www.nwhc.usgs.gov/disease_information/sylvatic_plague/index.jsp.

4. ALTERNATIVES INCLUDING THE PROPOSED ACTION

This section provides a description of reasonable alternative actions that address the Purpose and Need in sufficient detail to identify potential environmental impacts. The No-Action Alternative is included as a baseline and for comparison (40 CFR 1508.9(b)).

4.1 Proposed action (Alternative 1)

4.1.1 Project objective and context

Up to three small, short-term field trials will be conducted over a one to two (1 to 2) year period to evaluate the safety of SPV for prairie dogs under field conditions at selected sites in Colorado. These studies will provide important information regarding the uptake and safety of SPV in wild prairie dogs and non-target animals. It will also provide a framework and foundation for future

studies examining the use and effectiveness of SPV to prevent wide-spread plague induced mortality of prairie dogs.

4.1.2 Proposed activities

The USGS-NWHC is proposing to conduct small, short-term field trials to demonstrate the consumption and safety of the SPV in wild prairie dogs and non-target animals under field conditions. Cooperating agencies include CPW and Bureau of Land Management (BLM). Field trials will begin in free-ranging prairie dogs (Gunnison's, black-tailed, and/or white-tailed) in the summer of 2012. These trials will be designed to confirm biosafety and the dynamics of bait uptake in the field. Specific objectives are to measure bait uptake by target and non-target animals under relatively controlled field conditions, to demonstrate "safety" (i.e. absence of vaccine-associated pathology) primarily in non-target animals, and to evaluate the immunological response of target animals (i.e., serological evidence of vaccine exposure and perhaps challenge of a subset of apparently-vaccinated individuals in a laboratory setting). Selected sites will be limited in size (e.g., 20–50 acres) and access, will be dusted with insecticide (deltamethrin) to prevent coincidental epizootic plague during the course of the field trials, and will be amenable to contingency management in the unlikely event of an adverse outcome of vaccine uptake in non-target species. Control sites that deploy baits without vaccine will also be included. Relatively high bait distribution rates (53 baits/acre) will be employed to allow maximum contact and uptake by individual animals (Tripp et. al., unpublished). Animals captured after vaccine/bait distribution will be examined for evidence of vaccine uptake (through presence of biomarker in hair samples), signs of pox lesions and/or morbidity, and any carcasses found will be submitted for full diagnostic testing. Animals will be released or euthanized depending on their health status, as detailed in Section 4.1.3.1. If adverse effects are found or suspected in any species, laboratory studies will be conducted to more fully assess and characterize the health effects of the vaccine in the species involved, and the field study will be suspended until laboratory studies are completed.

Elements of the design for these studies will include:

- 1) Assessment of vaccine uptake via Rhodamine B biomarker and estimate of bait removal rate;
- 2) Comparison of an index of prairie dog abundance before and after vaccine distribution on both vaccinated and paired control colony sites;
- 3) Assessment and comparison of an index of other small rodent species abundance (and possibly survival) before and after vaccine distribution on both vaccinated and control sites;
- 4) Assessment of exposure to vaccine antigens via serology;
- 5) Post vaccination monitoring for pox lesions and mortality in both prairie dogs and non-target rodents (and other species);
- 6) Euthanasia and sampling of a limited number of non-target rodents for histopathologic evaluation;
- 7) Assessment of the presence of natural orthopox virus infections in free-ranging prairie dogs (a potential source of confoundment or interference);
- 8) Collection and assessment of bait-laden feces for vaccine shedding (not anticipated based on laboratory studies –Rocke, unpublished data); and

- 9) Molecular characterization of any poxvirus isolated to ensure no genetic changes occurred (not anticipated based on laboratory studies –Rocke, unpublished data).

4.1.3 Monitoring and mitigation activities

4.1.3.1 Monitoring

Prior to vaccine or control bait distribution, visual counts of prairie dogs as well as counts of active and inactive burrows will be conducted to determine the baseline pre-treatment prairie dog abundance index and activity level.

Trapping of prairie dogs and other small mammals will also be done prior to treatment to determine baseline pre-treatment small mammal diversity and abundance index and baseline pre-treatment prairie dog abundance index. All captured individuals will be ear-tagged or PIT (passive integrated transponder)–tagged to create a population of marked individuals that can be recaptured post-treatment for comparison and survival estimation.

Once baits have been distributed, they will be monitored once every 24 hours to determine the rate of removal. The monitoring of bait removal will continue until 90-100% of the baits have been removed or until seven (7) days have passed. Baits containing Rhodamine B are a bright red color making them easily visible in the field. After seven (7) days any remaining baits will be located and removed from the study site.

Bait uptake can be measured by incorporating a biomarker, Rhodamine B, into the bait (Fernandez and Rocke, 2011). After bait consumption, Rhodamine B can be visualized under natural light visible as red staining and under UV light as an orange fluorescence. Using microscopy, fluorescent bands can be detected in hair and whisker samples taken from animals that consumed the biomarker-laden bait.

One day after bait distribution, visual counts of prairie dogs and counts of active and inactive burrows will be resumed for comparison to the baseline pre-treatment prairie dog abundance index and activity level. During the post-vaccination visual counts, specific observations of prairie dog health and activity will be conducted. Burrow counts will again be conducted seven to ten days after bait distribution. The number and condition of burrows will be recorded and burrow activity data will be compared to the pre-treatment data.

The post-vaccination capture and recapture (of previously marked individuals) rates will be compared to the pre-treatment and control plot capture data to assess if prairie dog abundance or survival decreased after vaccination. Three to seven days after bait distribution, prairie dogs will be captured using live traps. Each captured prairie dog will be inspected for lesions consistent with poxvirus infection as well as other outward signs of a negative response to the vaccine such as lethargy, ataxia, tremors, nasal or ocular discharge, and unkempt appearance. Any prairie dogs or non-target species with these signs or suffering severe injury or morbidity will be humanely euthanized as detailed in Section 6.1.1 and their carcasses submitted to NWHC for necropsy and complete virologic and histologic examination.

Seven days after bait distribution, non-target small mammals will be captured using live traps. Sample collection, health inspections, and bait uptake comparisons between the pre/post-treatment and control plots will be conducted. The post-vaccination capture and recapture (of

previously marked individuals) rates will be compared to the pre-treatment and control plot capture data to determine if small mammal abundance or survival decreased after vaccination. Each captured small mammal will be inspected for lesions consistent with poxvirus infection as well as other outward signs of a negative response to the vaccine such as lethargy, ataxia, tremors, nasal or ocular discharge, and unkempt appearance. Any small mammal with these signs or suffering severe injury or morbidity will be humanely euthanized as detailed in Section 6.1.1 and their carcasses submitted to NWHC for necropsy and complete virologic and histologic examination.

4.1.3.2 Mitigation activities

Mitigation measures are any features of an action that serve to prevent, reduce, or compensate for impacts that otherwise might result from that action. Mitigation activities would include

- Public information and education actions and media announcements to inform the public about SPV bait distribution activities before they occur;
- Study description, including telephone numbers to call for more information, will be posted on signs at the study sites;
- Methods used to capture animals would be limited to cage traps for the most part. Animals caught in cage traps that must be sacrificed (killed) for testing would be euthanized in accordance with recommendations by CPW ACUC protocols;
- All drug use in capturing and handling animals would be under the direction and authority of the CPW veterinarian; and
- A contingency management plan will be in place in the unlikely case of an adverse event defined as widespread mortality or morbidity of prairie dogs or non-target species. This plan includes the use of toxicants to quickly control the prairie dog and/or non-target populations on the treatment plots.

4.2 Alternatives

4.2.1 Rationale behind selection of alternatives

Viable alternatives must enable collection of data to assess the safety of SPV in the field prior to further studies on vaccine efficacy and ultimate use of SPV as a management tool.

4.2.2 Alternative action—another time (Alternative 2)

This action would be to conduct the proposed studies at an alternative (later) time. The proposed time (summer 2012) is the earliest time when these studies would be possible, pending vaccine regulatory approval. Participating scientists are currently prepared to undertake the studies at the proposed times. If the studies are postponed until a future time, considerable delays in obtaining data assessing field safety of SPV would occur. This delay would impact future studies on field efficacy of SPV and its subsequent use as a management tool for prairie dog conservation and recovery of the black-footed ferret. Plague would remain a threat to these populations of animals during the intervening time with the potential for species of prairie dogs to become listed as threatened or endangered species.

4.2.3 Alternative action—other locations (Alternative 3)

In the event that the prairie dog colonies identified in Alternative 1 are unavailable for use due to reasons such as plague epizootics, lack of approval by landowners, or other explanations, substitute locations of suitable prairie dog colonies would need to be identified. This action

would delay the field studies as a result of additional time spent identifying suitable prairie dog colonies, obtaining permission from landowners, and holding public meetings to inform the public in the area. As in Alternative 2, delays in the proposed studies would impact future studies on field efficacy of SPV and its subsequent use as a management tool for conservation of prairie dogs and recovery of the black-footed ferret.

4.2.4 No action (Alternative 4)

No vaccine-laden bait or insecticide would be applied to prairie dog colonies. USGS and CPW would not conduct research for plague control or use resources available. Field studies assessing vaccine efficacy would be prevented. Sylvatic plague would continue to pose an unregulated threat to existing populations of prairie dogs in the study region.

4.2.5 Alternatives considered but eliminated from detailed analysis

The only alternative to field studies would be laboratory studies to assess the safety of the vaccine. Laboratory studies have already shown SPV to be safe in prairie dogs (Mencher et al., 2004; Rocke et al., 2008b, 2010a, unpublished data). Studies of other RCN-vectored vaccines have shown them to be safe in numerous species of animals (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a). Additional laboratory studies of SPV would not assess safety of the vaccine under field conditions and are, therefore, not considered further.

5. AFFECTED ENVIRONMENT

This section presents descriptive information on the environment of the areas that would be affected by the proposed action. Prairie dog colonies selected for the field studies would be in isolated areas with restricted access. Prospective study areas for Gunnison's prairie dogs in Colorado include colonies on state (CPW) property in the Gunnison basin. Prospective study areas for black-tailed prairie dogs include colonies located on isolated, uninhabited natural areas that are not federally owned in northeastern Colorado. Prospective study areas for white-tailed prairie dogs may include non-federal lands located in northwestern Colorado.

The proposed action does not involve construction, major ground disturbance, or habitat modification. Therefore, the following resource values are not expected to be affected by the proposed action: soils, geology, minerals, water quality/quantity, visual resources, air quality, prime and unique farmlands, aquatic resources, vegetation, and range. These resources will not be analyzed further.

The proposed action will have negligible, if any, effects on the surrounding communities, including minority and low-income populations. Study sites will be approximately 10 miles from the nearest towns (Gunnison and Almont for Gunnison's prairie dog site; Wellington and Carr for black-tailed prairie dog site; undetermined at this time for white-tailed prairie dog site). The populations of these towns range from 300 to 5,300 with >85% of residents identifying themselves as white. Field studies will be conducted on isolated sites closed or restricted to the public. For sites on privately owned land, studies will be undertaken with landowner permission.

5.1 Physical Description and Climate

Prospective study areas for Gunnison's prairie dogs include colonies on CPW (Miller Ranch SWA) and BLM owned land. The primary study colonies are located on open space

approximately 10 miles north of Gunnison, Colorado. The treatment colony is approximately 30 acres and is located on the Miller Ranch SWA (Figure 2) which is closed to the public. The control colony (no vaccine) is approximately 30 acres and is located on BLM land (Kenny Moore colony). The primary study sites are separated by a distance of approximately two (2) miles and are primarily used for cattle grazing and wildlife conservation. The study sites are at an elevation of 8,329 feet and get 16 inches of rain per year. Average snowfall is 106 inches with a low in January of -3 °F and an average high of 79 °F in July.

Prospective study areas for black-tailed prairie dogs include a complex of colonies located on land owned by the City of Fort Collins Natural Areas Program (Soapstone Prairie) and the City of Fort Collins Utilities Department (Meadow Springs Ranch). These colonies are located on isolated open space in northern Larimer County approximately 30 miles north of Fort Collins, Colorado (Figure 3). These lands are primarily used as natural areas and are closed to the public or will have restricted public access during the study. The primary treatment colony “MSR 14” is approximately 55 acres in area and the primary control colony “MSR 16” is approximately 40 acres in area. These colonies are the primary study sites and are separated by a distance of approximately two (2) miles. Additional colonies within this complex may be selected as study sites if the primary sites are not suitable at the time the study is initiated. Plague epizootics, grazing schedules, and local weather conditions may influence the selection of the study colonies within this complex. Grazing will be prohibited at the selected colony site during the study. The colonies within this complex are at an elevation of 5,715 feet and get 13 inches of rain per year. Average snowfall is 38 inches with a low in January of 11 °F and an average high of 89 °F in July.

Specific study areas for white-tailed prairie dogs have not been finalized at this time. Future study areas may include the Wolf Creek management area (BLM) and private lands with conservation easements with the CPW located in southern Moffat County and northern Rio Blanco County, Colorado. These areas are primarily used for cattle and sheep grazing. Potential study sites in Moffat County are at an elevation of 5,917 feet and get 12 inches of rain per year. Average snowfall is 61 inches with a low in January of 2 °F and an average high of 87 °F in July.

Figure 2. Map of the Study Area on the Miller Ranch State Wildlife Area - Gunnison County

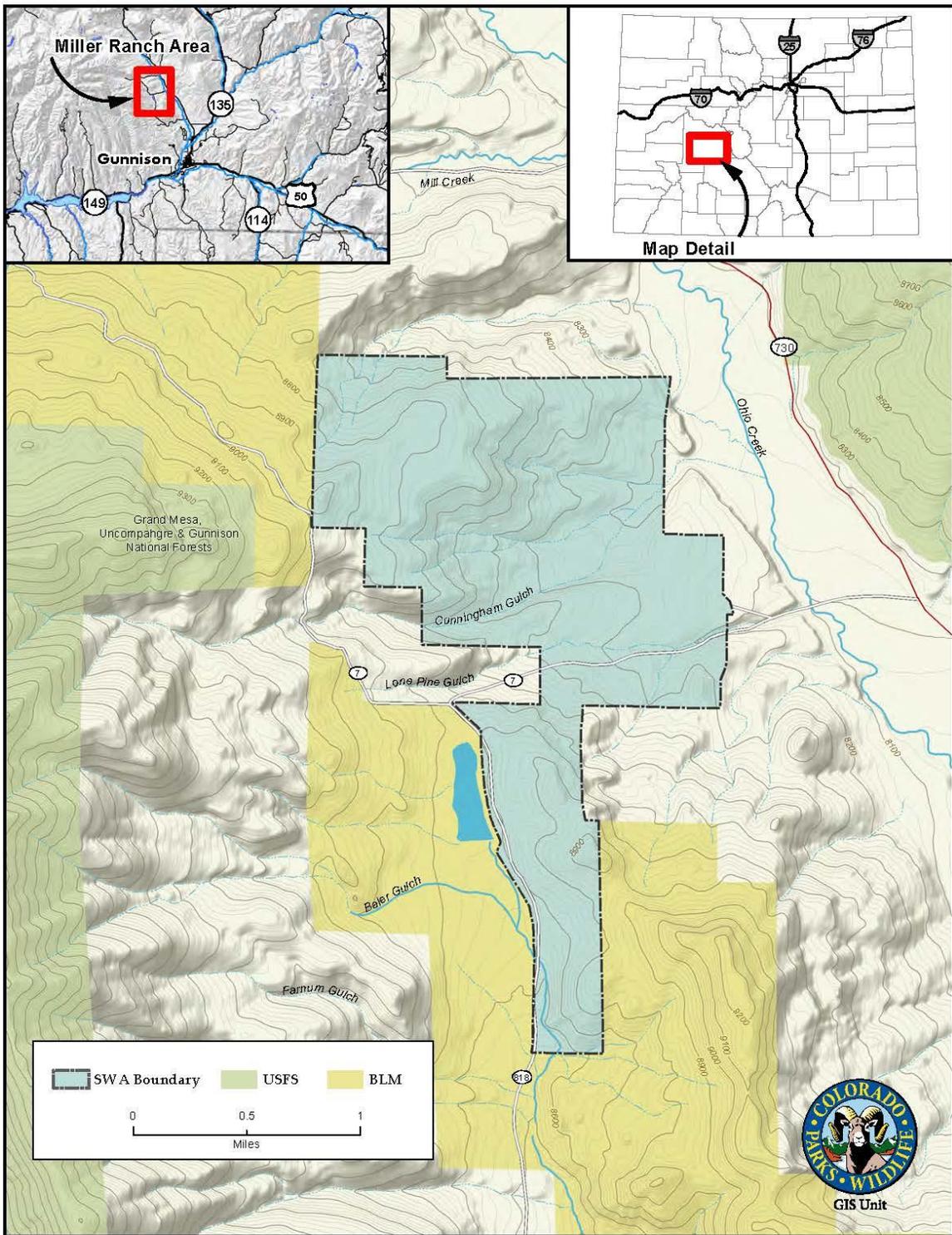
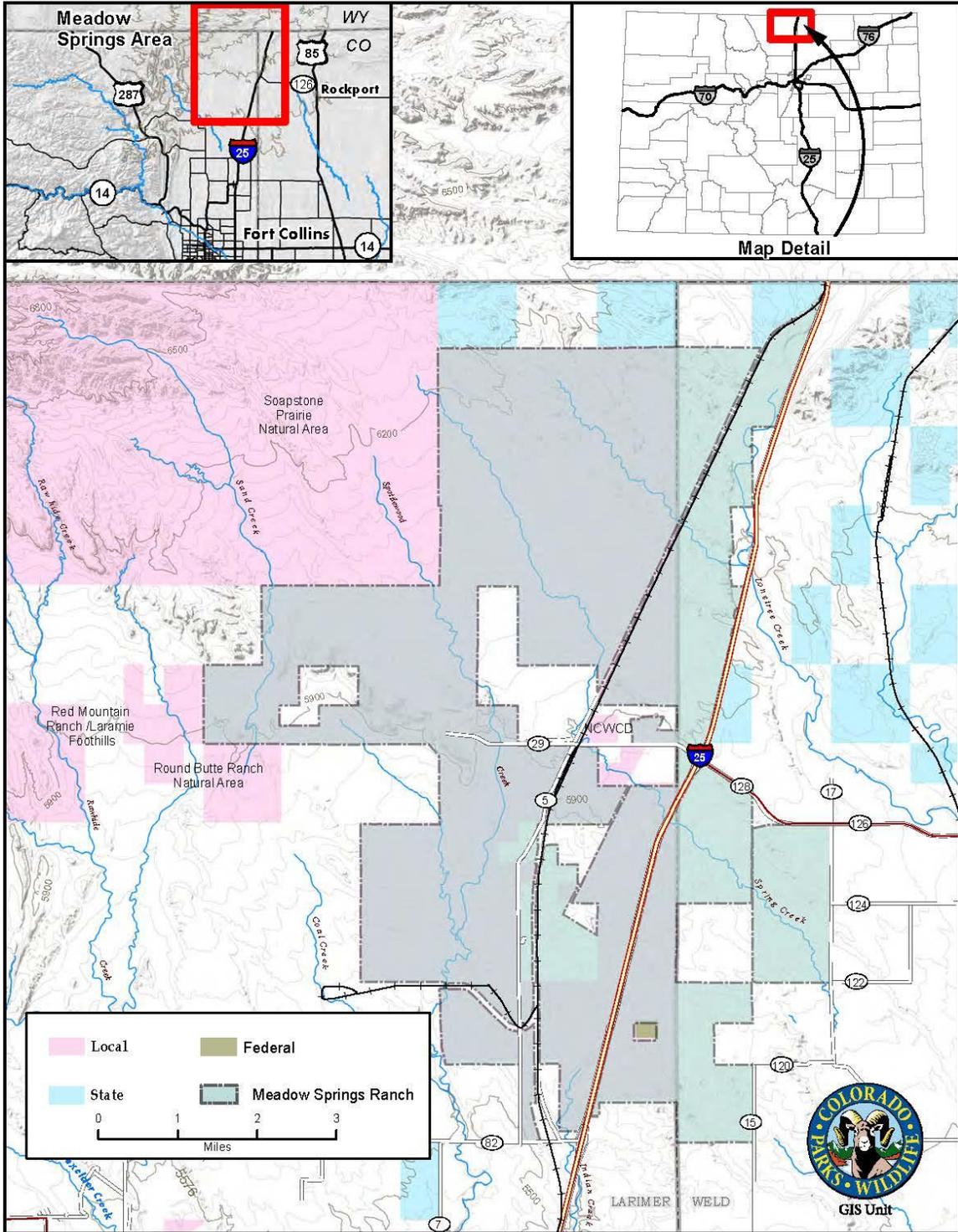


Figure 3. Map of the Study Area on the Soapstone Prairie Natural Area and Meadow Springs Ranch – Larimer County



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5.2 Biological Resources

5.2.1 Terrestrial Vegetation

Black-tailed prairie dog colonies are found in shortgrass or mixed prairie consisting primarily of buffalo grass (*Buchloe dactyloides*), blue grama (*Bouteloua gracilis*), Western wheatgrass (*Pascopyrum smithii*), Russian thistle, pigweed, and ragweed.

White-tailed prairie dog colonies are found in open shrublands and semidesert grasslands consisting primarily of grasses, forbs, and low shrubs such as sagebrush or saltbrush.

Gunnison's prairie dog colonies are found in grasslands and semidesert and montane shrublands consisting of grasses, forbs, and low shrubs such as sage and rabbitbrush.

5.2.2 Terrestrial Mammals

Rodent species include prairie dogs (black-tailed, Gunnison's, and white-tailed), deer mice (*Peromyscus maniculatus*), northern grasshopper mice (*Onychomys leucogaster*), chipmunks (*Tamias* spp.), and voles (*Microtus* spp.). Other small mammals include shrews (*Sorex* spp.), rabbits, and spotted skunks. Predators include badgers, swift foxes, coyotes, weasels, and bobcats. Ungulates include domestic cattle and sheep, deer, pronghorn, and elk.

5.2.3 Birds

Numerous species of birds are found on the study sites, including hawk, eagle, curlew, shrike, sparrow, bunting, lark, meadowlark, longspur, burrowing owl, and grouse.

5.2.4 Arthropods

Numerous species of insects are found on the study sites including, among others, fleas, flies, ants, butterflies and moths, beetles, bugs, bees, grasshoppers, and crickets. Non-insect arthropods include spiders and ticks.

5.2.5 Reptiles and Amphibians

Reptiles found on the study site include snakes and lizards. Amphibians include spadefoot toads.

5.3 Federal Threatened and Endangered Species and Critical Habitat

The following threatened, endangered, and candidate species may be present during prairie dog and small mammal capture:

Gunnison County: Gunnison-sage grouse, Gunnison's prairie dog, and

Moffat County: Greater Sage-grouse.

The USFWS (USFWS, 2012) indicated that no federally listed or proposed, threatened, or endangered species or critical habitat under jurisdiction of the USFWS are known to occur in the proposed project areas (Appendix B, page B3-B4).

5.4 Cultural Resources

Cultural resources in the study sites relate to historic occupation of these areas by Native Americans and may include human remains and associated artifacts. The Colorado State Historic Preservation Officer (March 2012) has determined that no historic properties will be affected at the study sites (Appendix B, page B1-B2).

5.5 Human Uses

5.5.1 Subsistence Uses

The study sites are primarily used for cattle and sheep grazing.

5.5.2 Other Public Uses

Study areas on some BLM lands (control sties that do not receive vaccine) may have recreation uses such as prairie dog shooting.

5.6 Designated Wilderness

There are no designated wilderness areas in the study sites.

6. ENVIRONMENTAL EFFECTS

6.1 Issues considered

6.1.1 Issues considered in detail

The impacts of the four (4) alternatives on the natural environment of the study sites are analyzed with respect to the vaccine, biomarker, insecticide, and capture/handling of animals.

- Vaccine

SPV uses a recombinant raccoon-pox (RCN) virus incorporating two genes of *Yersinia pestis*. The vaccine is orally delivered to target animals via edible baits composed of food grade ingredients and contain potassium sorbate (<2% concentration) as a preservative and Rhodamine B (<0.5% concentration) as a biomarker. The vaccine virus produces proteins of *Yersinia pestis*, F1 and V307, in infected cells to stimulate an immune response by prairie dogs that consume vaccine-laden baits.

- Biomarker

Biomarkers are distinctive biological indicators used to identify, often through indirect means, when an event or physiologic process of interest has occurred in an animal. Biomarkers are normally incorporated into the baits to identify animals that have consumed vaccine-laden bait. SPV baits contain Rhodamine B, an industrial and analytical dye that has been widely used as a marker and tracer in animal studies that marks hair, feces, or blood (Evans and Griffith, 1973; Johns and Pans, 1981; Lindsey, 1983; Fisher et al., 1999). After bait consumption, Rhodamine B can be visualized under natural light (red staining) and under UV light (orange fluorescence). Under fluorescence microscope, fluorescent bands can be detected in hair and whiskers removed from captured animals. SPV baits will contain 0.35% Rhodamine B biomarker.

- Insecticide

Deltamethrin, as the active ingredient of DeltaDust® (0.05%), is an insecticide that provides broad spectrum and residual control of crawling arthropods and is labeled for the control of fleas in rodent burrows. It has been shown to be effective in controlling fleas and thus the spread of plague throughout prairie dog colonies (Seery et al., 2003; Cully et al., 2006). Deltamethrin is a granular insecticide delivered by a wand as deep as possible into rodent burrows and then rotated to coat the perimeter soil. The dust then coats the rodents when they enter and exit the burrow system. The intent of a dusting effort is to reduce flea populations, as the major known vector of plague, in prairie dog colonies to reduce the risk of a plague outbreak during the proposed field studies. Treatment would consist of individually metering 4-5 g of product (0.002 g active

ingredient) into the entrance of prairie dog burrows with shoulder-carried application equipment. Application would be conducted by, or under the direct supervision of, CPW Wildlife Health Program staff in accordance with rules in Colorado. Treatment activities would occur over a two to three (2-3) week period between June and October 2012, and March and October 2013, by personnel appointed by the CPW.

- **Capture and handling**

Prairie dogs and small mammals will be captured using Tomahawk or Sherman live traps. For prairie dogs, traps will be baited with sweet horse feed and set between 5:00 a.m. and 7:00 a.m. Traps will be left open and checked every one to two (1 to 2) hours until temperatures reach 26°C (78.8°F) or inclement weather such as rain or high winds interfere with trapping efforts. Traps will not be set if inclement weather is forecast. Field technicians will remain at or near the trapping site while traps are open to ensure animals are released and all traps are closed in inclement weather. For nocturnal rodents, Sherman traps will be opened in the late evening unless inclement weather is forecast. Nocturnal species will be removed from traps at first light the next morning, which usually requires one to two (1-2) hours for processing. Thus, animals captured in the early evening will be in the traps for as short a time as possible, usually <12 hours.

Prairie dogs and small mammals will be anesthetized using isoflurane vaporizers with an oxygen flow rate of 1.5–2 liters per minute, inducing with 3–4 % isoflurane and maintaining to effect usually around 1–2%. After processing, animals will be held in traps in the shade to allow full recovery following anesthesia. Once recovered, animals will be released at the point of capture. If an animal is severely injured and cannot be released or is obviously suffering from severe disease (plague) it will be euthanized following the ACUC recommendations for small mammals over 500 g (Wild et al., 1992). Acceptable euthanasia procedures will include anesthetic overdose or cervical dislocation while under anesthesia. Regardless of the method of euthanasia used, to assure that death occurs, we will perform cervical dislocation, in which the spinal cord is separated from the brain by applying pressure to the neck and dislocating the spinal column from the skull or brain.

6.1.2 Issues not considered in detail with rationale

- **Bait composition**

The edible peanut butter baits are composed of food grade ingredients and contain potassium sorbate (<2% concentration) as a preservative. All ingredients are non-toxic.

- **Potential impacts on threatened and endangered species**

Threatened and endangered species are not expected to be impacted by this work. Gunnison's sage-grouse and greater sage-grouse are rarely encountered in the study areas. Traps will be baited with baits that are unattractive to grouse. If threatened or endangered species are encountered during our study, trapping will be suspended and further efforts will be made to avoid the incidental capture of and to minimize disturbance to these species. In the past, no incidental capture of threatened and endangered species has occurred when trapping has been done in similar habitats. All-terrain vehicles will not be used and all work will be conducted on foot. Field crews will be trained to identify all threatened and endangered plants, mammals, and

birds and to avoid them if discovered. CPW biologists would integrate measures necessary to avoid disruption of grouse reproductive functions.

- Potential impacts on cultural resources

The proposed action would not cause major ground disturbance, would not cause any physical destruction or damage to property, or any alterations of property, wildlife habitat, or landscapes, and does not involve the sale, lease, or transfer of ownership of any property. Also, the proposed methods do not have the potential to introduce visual, atmospheric, or audible elements to areas in which they are used that could result in effects on the character or use of historic properties. Any cultural artifacts discovered during the study will be left undisturbed.

- Human subsistence and other uses

Baits and vaccine will not be distributed in areas that are actively grazed. Livestock will be removed from the study sites before safety trials begin. Prairie dog shooting will be prohibited while studies are conducted. Impacts on human subsistence and other uses will be negligible.

- Potential human health impacts in the event of human consumption of vaccinated wildlife

The issue expressed here is the potential to develop a raccoon pox infection from eating a vaccinated prairie dog or some other animal that has eaten one or more SPV baits. Prairie dogs are sometimes eaten by Native Americans.

Biophotonic imaging studies showed that RCN exposure via the oral route in prairie dogs resulted in a localized infection that did not progress systemically (Berlier et al., 2010). The RCN virus from SPV would only bind to animal tissues in the mucous membranes of the oral cavity, pharynx, and esophagus when orally ingested since RCN does not spread throughout the body of the animal. Those particular tissues are rarely consumed by humans, but if they were, they would most likely be cooked which would kill the virus. In addition, public access to study sites will be restricted. Therefore, the potential for adverse health effects from consuming animals that have eaten SPV baits are low.

6.1.3 Effects of Global Warming, Habitat Loss, and Pollution on Wildlife Populations

Program activities likely to result from the proposed action would have a negligible effect on atmospheric conditions including the global climate. Meaningful direct or indirect emissions of greenhouse gases would not occur as a result of the proposed action. The proposed action would meet the requirements of applicable Federal laws, regulations, and Executive Orders (See Appendix C) including the Clean Air Act and Executive Order 13514. Other than minor uses of fuels for motor vehicles and other materials, there are no irreversible or irretrievable commitments of resources. The contribution of the proposed action to the emission of gases that potentially contribute to global warming will be similar to the other alternatives and is expected to be minimal. Thus, these will not be analyzed further.

6.2 Issues analyzed by alternative

6.2.1 Proposed Action (Alternative 1)

6.2.1.1 Potential impacts of vaccine

Based on preliminary bait distribution trials, the probability of exposure to the vaccine by ingestion of baits is high for prairie dogs and other rodents; they are known to consume the baits (Tripp, unpublished data). Non-target animals, other than rodents, that may encounter vaccine-laden bait include ungulates, canids, felids, badgers, raccoons, skunks, birds, and reptiles. For these animals, the probability of ingesting baits is low to moderate. Over 12,000 camera-hours of field monitoring and recent experiences with captive native ungulates (elk, mule deer) have shown that the bait is not attractive to wild ungulates (pronghorn, mule deer, elk) or to wild carnivores (fox, coyote, badger, skunk) and is generally unpalatable to captive hoofed stock, even those habituated to eating treats (Miller and Tripp, unpublished data). Domestic dogs also generally found the bait unpalatable (Miller and Tripp, unpublished data).

The likelihood of inadvertent human exposure to vaccine-laden baits during conduct of this field trial is extremely limited. Study personnel distributing the baits will be trained in proper handling of the baits and will wear personal protective equipment (latex gloves). Uneaten baits will be collected after seven (7) days and disposed of by autoclaving. Human exposure will be further limited by the restricted access to the remote study sites, the anticipated pre-field trial publicity, and the short time the baits will remain in the field.

The virulence of RCN is highly attenuated by inactivation of the thymidine kinase (*tk*) gene by insertional recombination. Recombinant RCN has been used in several oral vaccines in raccoons, mice, cotton rats, rabbits, striped skunks, dogs, bobcats, non-human primates, cats, and sheep with no harmful effects (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a).

6.2.1.1.1 Potential to cause plague

The nature of the recombinant virus used as SPV is such that it cannot cause plague. SPV carries only two genes associated with virulence of *Yersinia pestis*. In SPV (RCN-F1-V307) designed for use in prairie dogs, the entire gene for F1, a capsular protein, was inserted. For the V gene, a C-terminally truncated portion was inserted that expressed only 307 amino acids (instead of the 326 of the full V gene) (Rocke, et al., 2010b). Although animals vaccinated with full V antigens do not show adverse reactions, a truncated V gene (V307) was designed to remove a portion of the V gene that was shown by other investigators to have immunosuppressive effects in laboratory studies (Overheim et al., 2005). SPV does not contain genes of *Yersinia pestis* necessary for production of the bacteria which would need to occur to produce plague.

F1 and V antigens of *Yersinia pestis* have been effectively and safely used in prairie dogs (Mencher et al., 2004; Rocke et al., 2008b, 2010a, unpublished data), mice (Heath et al., 1998; Williamson, 2001; Osorio et al., 2003b; Rocke et al., 2010b), and black-footed ferrets (Rocke et al., 2004a, 2006, 2008a) to protect them against plague.

6.2.1.1.2 Potential to cause raccoon pox

RCN is considered a BSL-2 pathogen. The deletion of the *tk* gene considerably attenuates the virus. The virulence of the recombinant RCN is expected to be low in prairie dogs, non-target

animals, and humans. Recombinant RCN viruses have been developed as vaccines for rabies (Esposito et al., 1988, 1989, 1992), feline panleukopenia virus (Hu et al., 1996), and plague (Osorio et al., 2003b; Mencher et al., 2004; Rocke et al., 2008a, 2010ab; Rocke, unpublished) using the *tk* gene as the site for insertional recombination. Recombinant RCN has been used in several oral vaccines in raccoons, mice, cotton rats, rabbits, striped skunks, dogs, bobcats, non-human primates, cats, and sheep with no harmful effects (Esposito et al., 1988, 1989, 1992; Fekadu et al., 1991; DeMartini et al., 1993; Osorio et al., 2003a). Based on outcomes of other RCN vaccine studies, ingestion of vaccine-laden baits is not expected to cause adverse reactions in non-target animals and humans.

In experimental studies, there have been no adverse effects associated with the use of oral RCN-vectored plague vaccines in prairie dogs (Mencher et al., 2004; Rocke et al., 2008b, 2010a). USGS has injected or fed animals with RCN plague vaccine constructs and compared results with both wild type RCN virus and *tk*⁻RCN virus. No morbidity or mortality has been observed from any of these viruses in either mice or prairie dogs (Rocke et al., 2010ab). No mortality has been observed even in severe combined immune deficient (SCID) mice upon injection of RCN-based vaccines.

The only documented case of human exposure to recombinant raccoon poxvirus occurred as a result of a laboratory needlestick accident with an experimental plague vaccine using recombinant raccoon poxvirus as a vaccine vector for *Y. pestis* antigen F1 (Rocke et al., 2004b). Within nine (9) days, the patient developed a small blister at the injection site that healed within four (4) weeks; no other systemic symptoms were reported during this period. Raccoon poxvirus was cultured from the lesion, and the patient developed antibody to plague antigen (F1) and RCN suggesting infection of the patient with RCN-F1. The blister was possibly due to an inflammatory response to limited viral replication.

6.2.1.1.3 Potential for recombinant RCN to revert to virulence or to recombine with other viruses in the wild and result in a virus that could cause disease in humans or animals

The recombinant raccoon poxvirus used in the vaccine is expected to be genetically stable so that it would not become virulent after it replicates in animals that eat SPV baits with the potential of being passed on to other animals. The *tk* gene (approximately 844 bp) of RCN has been inactivated by insertional recombination with IRES/tPA sequences and the F1 and V307 genes of *Yersinia pestis*. The presence of these genes is not known to promote any homologous recombination or DNA insertion. Because of the large insertion made into the *tk* gene, it is unlikely that the recombinant RCN virus would regain its *tk* gene to become fully virulent. In studies of *tk*⁻ strains of vaccinia, a related poxvirus, no evidence of reversion was detected (Buller et al., 1985). In addition, there is no evidence to suggest that the donor DNA sequences (F1 and V307) enhance the virulence of RCN or its ability to survive in target animals.

A back-passage study involving an initial passage of RCN-F1-V307 and two back-passages of pooled virus isolates in prairie dogs demonstrated that RCN-F1-V307 can be shed orally by inoculated prairie dogs but does not pass between prairie dogs (Rocke, unpublished). None of the prairie dogs developed clinical signs associated with infection of raccoon poxvirus showing

the virus did not revert to virulence. These results indicate that although RCN-F1-V307 is replicative in prairie dogs it is not pathogenic.

6.2.1.2 Potential impacts of biomarker

The lethal dose 50% (LD50) of Rhodamine B in orally inoculated laboratory mice is 887 mg/kg (Rhodamine B; MSDS, 2007). Each bait used in this study would be approximately 4 g and contain 0.35% Rhodamine B (14 mg/bait). If a 1 kg prairie dog consumes one full bait, the dose would be 14 mg/kg. A 1 kg prairie dog would have to consume over 60 baits to reach the LD50. Laboratory observations have shown that prairie dogs rarely consume more than six (6) baits when given unlimited access (Fernandez and Rocke, 2011). In the field, it would be unlikely that individual prairie dogs would encounter more than six (6) baits given the bait distribution design for this project; baits will be distributed by hand along transects spaced 10 meters (33 feet) apart at a bait density of 53 baits/acre or 1,060 baits per 20 acre plot.

Each non-target species would have to ingest 60 baits for every kilogram of body weight to reach the LD50. For example, a 20-40 g mouse would need to ingest 5.2 – 10 g of baits with Rhodamine B at one time to reach an LD50. That would be one-fourth of its body weight at one time. Based on a daily food intake value of 15 g/100 g body weight, a 20-40 g mouse would ingest 3-6 g of food daily. This would be equivalent to 0.75-1.5 baits/day which is less than the LD50. In the field, it would be unlikely that individual mice would encounter and consume more than 1 bait/day given the bait distribution design for this project. An 18 kg coyote would need to ingest 1,080 baits at one time to reach an LD50, a number greater than the total number of baits distributed in the 20 acre study plot. Based on these measurements, it is not expected that baits using biomarkers would impact terrestrial wildlife populations in the study areas. In addition, baits are not palatable to ungulates and carnivores.

The toxicity of Rhodamine B is so low that it would be virtually impossible for a domestic cow or sheep to consume sufficient baits at the rate proposed to suffer any ill effects. A 640 kg cow would need to ingest more than 38,000 baits at one time; a 125 kg sheep would need to ingest 7,500 baits at one time.

Although, the LD50 of Rhodamine B in birds has not been reported, ingestion of up to 30 mg/kg Rhodamine B by domestic chickens showed no toxic effects (Lindsey, 1983). For raptors, the probability of ingesting SPV baits is low. If a 1.5 kg raptor were to eat 45 mg of Rhodamine B, or more than three (3) SPV baits, no toxicity would be expected.

Raptors and other predators may consume rodents that have ingested baits, although it is not expected that this source will lead to a dangerous level of Rhodamine B ingestion. Feces from raptors and coyotes became dyed by Rhodamine B after feeding on prey that was exposed to 1% or greater concentration of Rhodamine B. No adverse effects were noted (Evans and Griffith, 1973).

6.2.1.3 Potential impacts of insecticide

Deltamethrin, as the active ingredient of DeltaDust® (0.05%), is an insecticide that provides broad spectrum and residual control of crawling arthropods. Toxicity for birds is very low (LD50 range of 5,000-10,000 mg/kg) and practically nontoxic to mammals (LD50 range of

6,500-22,000 mg/kg). There is no information suggesting that deltamethrin has any tendency to bioaccumulate in animal tissues and the chemical has been determined to be noncarcinogenic and have no deleterious effects (<http://www.ace.orst.edu/info/extoxnet/pips/deltanet.htm>). Because the treatment and application is specifically directed at controlling flea populations in prairie dog burrows, the proposed application rate is about 150 times lower than recommended rates for customary home and agricultural use. These techniques have been shown to be effective at controlling fleas for six to eight (6-8) months (Tripp, et. al., unpublished; Biggins et al., 2010). Because the product would be placed down individual prairie dog burrows, it would remain essentially unavailable to terrestrial dwelling animals, including livestock and big game.

This proposal will not affect air or soil quality. No DeltaDust® would be applied above ground level; dust is applied only directly inside each burrow. Most of the compound is absorbed by the soils. This will have negligible effects on a landscape scale. No activities would occur within 50 feet of streams, so water would not be affected.

Human exposure to the insecticide would be limited. Application would be conducted by, or under the direct supervision of, CPW Wildlife Health Program staff. Product transport, mixing, application, storage, cleanup, and use of protective gear would be consistent with the label specifications. Access to the study sites would be restricted to limit other human exposures.

As used for this project, and as allowed on the pesticide product labeling, DeltaDust® is expected to kill fleas in prairie dog burrows and on mammals that use treated burrows. It is an unrestricted use pesticide and considered safe for many applications including use in and around homes. No sensitive insect species are associated with prairie dog colonies on the study sites. Therefore, use of this product will not cause decline of individuals or populations of sensitive species nor contribute negatively towards population trends.

The label for DeltaDust® requires avoidance of applications to water bodies. The study sites have limited water bodies and the method of application (application at and around prairie dog burrows) would avoid any contact of the product with any bodies of water. A 50-foot buffer will be used around any body of water. Therefore, there would be no effect on any aquatic organisms.

The proposed project is likely to reduce arachnid populations that inhabit prairie dog burrows that receive treatment. No arachnid species in the study sites are considered rare or declining. Arachnid populations in the areas surrounding the study sites will have no potential for exposure to the treatment, which will leave adequate populations to re-inhabit prairie dog burrows when the effects of insecticide diminish which is expected to be six to ten (6-10) months following treatment.

Based on this information and analysis, the effects of insecticide on non-target wildlife from the proposed action would be inconsequential.

6.2.1.4 Potential impacts of capture/handling methods used in monitoring and surveillance actions

Trapping and handling of prairie dogs and non-target rodents will be conducted by experienced personnel. Traps will be checked frequently and animals released immediately after sample collection, resulting in little impact.

6.2.2 Alternative action—another time (Alternative 2)

This action would be to conduct the proposed studies at an alternative (later) time. Delaying the timing of the proposed project would not result in benefits for prairie dogs or non-target animals. Delay would potentially harm the prairie dog colonies if a plague outbreak were to occur during the intervening time. Other species that depend on prairie dog colonies could also be negatively impacted. Alternative study sites would need to be selected if plague outbreaks occurred in the proposed sites. Delays in obtaining data assessing the field safety of SPV would impact future studies on field efficacy of SPV and its subsequent use as a management tool for conservation of prairie dogs and recovery of the black-footed ferret. Plague would remain a threat to these populations of animals with the potential for species of prairie dogs to become listed as threatened or endangered species.

6.2.3 Alternative action—other locations (Alternative 3)

Alternative sites identified would be similar to those described in Section 5, in that they would have restricted access and comparable biological resources, cultural resources, and human activity. Thus, the potential impacts of the vaccine, biomarker, insecticide, and capture and handling methods used in monitoring and surveillance actions on the alternative sites would be similar to those described for Alternative 1, the preferred option. As mentioned previously, this action would delay the field studies leading to the negative effects associated with Alternative 2.

6.2.4 No action alternative (Alternative 4)

Under the no action alternative, no proposed actions would take place and would have no impact on terrestrial wildlife or humans as a direct result. No adverse effects from vaccine, biomarker, or insecticide would occur. However, prairie dogs and other species will be negatively affected by outbreaks of plague. SPV would be unavailable as a management tool to combat plague.

6.3 Cumulative Impacts

No cumulative environmental impacts are expected from any alternative, with the possible exception of Alternative 4 - No Action, which might lead to increased plague activity in prairie dogs. The analysis in this EA indicates that the proposed short-term field trials will not result in risk of cumulative adverse impacts on the quality of the human environment.



Photo courtesy of Dean Biggins, USGS

6.4 Summary of impacts of alternatives for each issue

| Issue/Impact | Alternative 1 Proposed Action | Alternative 2 Another Time | Alternative 3 Other Locations | Alternative 4 No Action |
|--|---|--|--|--|
| Potential to cause plague. | No risk for humans or animals. | No risk from SPV for humans or animals. Risks of naturally occurring plague may be higher in animals and humans during time before postponed use of SPV. | No risk from SPV for humans or animals. Risks of naturally occurring plague may be higher in animals and humans during time required to identify and coordinate alternative locations. | No risk from SPV. Risk of naturally occurring plague occurring in prairie dogs may be higher without protection from SPV. Risk to humans may be higher if plague epizootics occur. |
| Potential to cause raccoon pox. | Based on outcomes of other RCN vaccine studies, ingestion of vaccine-laden baits is not expected to cause adverse reactions in non-target animals and humans. | Same as Alternative 1. | Same as Alternative 1. | No risk from SPV. |
| Potential for recombinant RCN to revert to virulence or to recombine with other viruses in the wild and result in a virus that could cause disease in humans or animals | The recombinant RCN has been inactivated. | Same as Alternative 1. | Same as Alternative 1. | No risk from SPV. |
| Impacts of biomarker. | Animals are not expected to ingest enough baits to reach the LD50. | Same as Alternative 1. | Same as Alternative 1. | No risk from SPV. |

| Issue/Impact | Alternative 1 Proposed Action | Alternative 2 Another Time | Alternative 3 Other Locations | Alternative 4 No Action |
|--|---|--|----------------------------------|--|
| Impacts of insecticide. | Deltamethrin is non-toxic to mammals and birds. Application of insecticide would be confined to prairie dog burrows limiting its availability to terrestrial animals and humans. Effects on populations of non-flea arthropods would not be long-lasting. | During the time lag, flea populations would not be reduced, potentially allowing plague epizootics to occur. | Same as Alternative 1. | No risk. Plague epizootics may occur due to active flea populations. |
| Impact of methods used to collect wild animal specimens critical for timely program evaluation. | Collections will be conducted by experienced personnel. Traps will be checked frequently and animals released immediately after sample collection. | Same as Alternative 1. | Same as Alternative 1. | No impact. |

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10. GLOSSARY

| | |
|--------------------|---|
| endemic | Commonly present within a human population or a geographical area. |
| enzootic | An animal disease that commonly is present within a population or a geographical area or pertaining to such a disease. |
| epizootic | An outbreak of disease affecting a greater number of animals than normal, typically involving many animals in the same region at the same time or pertaining to such an outbreak. |
| LD50 | (Lethal dose 50%). The dose of a substance that would kill one half of the test animals. |
| recombinant vector | Produced by the combining of genetic material from more than one origin. An insect or other living organism that carries and transmits a disease agent from one animal to another. |
| zoonotic | Pertaining to a disease that can be transmitted from animals to humans, and vice versa. |

Appendix A

Open Houses

Open House Plan

Sylvatic Plague Vaccine Research in Larimer and Gunnison Counties

- **February 15, 2012 from 4:00PM to 7:00PM MDT**
At the Leeper Center conference room, 3800 Wilson Ave. Wellington, CO 80549
- **February 16, 2012 from 4:00PM to 7:00PM MDT**
At the Aspinall-Wilson Conference Center at 909 E. Escalante Drive, Western State College, Gunnison, CO 81230
- Open House format
- Sign-in sheet
- Large map of area with potential study sites noted
- Posters with project information, frequently asked questions, and safety information
- Available to listen to input and answer questions:
 - * Concerning USGS-NWHC vaccine development and safety:
Dr. Tonie Rocke, USGS-NWHC
 - * Concerning proposed project:
Dan Tripp and Dr. Mike Miller, Colorado Parks and Wildlife
Eva Bryson, USGS
 - * Concerning environmental impacts:
Dan Tripp and Nate Seward, Colorado Parks and Wildlife
Daylan Figgs, Fort Collins Natural Areas Program
Ray Kemp, Fort Collins Utilities Department
Darren Long, Bureau of Land Management
- Several comment forms, pencils and a large container to hold them
- Refreshments

Open House Notice, Larimer County:

Open House Announcement

Colorado Parks and Wildlife, together with the U.S. Geological Survey, will be hosting an open house in Wellington on February 15th to provide information on a wildlife research project that will be conducted on the Soapstone Prairie Natural Area and Meadow Springs Ranch in northeastern Larimer County. The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife.

Representatives from Colorado Parks and Wildlife and USGS will be available to describe the project to the public and take questions regarding the proposed research as related to the environmental assessment process.

Location: Leeper Center conference room at 3800 Wilson Ave. Wellington, CO 80549

Date: February 15, 2012

Time: 4 p.m. to 7 p.m.

Format: Open House

USGS and CPW: www.nwhc.usgs.gov and www.wildlife.state.co.us

Contact: Dan Tripp (970) 472-4478 or dan.tripp@state.co.us

Open House Notice, Gunnison County:

Open House Announcement

Colorado Parks and Wildlife, together with the U.S. Geological Survey, will be hosting an open house in Gunnison on February 16th to provide information on a wildlife research project that will be conducted on the Miller Ranch State Wildlife Area in Gunnison County. The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife.

Representatives from Colorado Parks and Wildlife and USGS will be available to describe the project to the public and take questions regarding the proposed research as related to the environmental assessment process.

Location: Aspinall-Wilson Conference Center at 909 E. Escalante Drive, Western State College, Gunnison, CO 81230

Date: February 16, 2012

Time: 4 p.m. to 7 p.m.

Format: Open House

USGS and CPW: www.nwhc.usgs.gov and www.wildlife.state.co.us

Contact: Dan Tripp (970) 472-4478 or dan.tripp@state.co.us

Notice Sent To:

Print:

North Forty News: January 25 to February 15, 2012

Gunnison Country Times: February 2 to 9, 2012

Community Calendars hosted by these media

Online:

North Forty News online: January 30 to February 15, 2012

Gunnison Country Times online: February 2 to 16, 2012

Community Bulletin Boards:

Wellington Post Office: February 1 to 15, 2012

Wellington Library and Community Center: February 1 to 15, 2012

Wellington Grocery Store: February 1 to 15, 2012

Carr Post Office: February 1 to 15, 2012

Fort Collins CPW Office: February 2 to 15, 2012

Gunnison CPW Office: February 2 to 16, 2012

Gunnison BLM Office: February 6 to 16, 2012

Gunnison U.S. Forest Service/NRCS office: February 6 to 16, 2012

Western State College: February 6 to 16, 2012

Adjacent Landowners and Stakeholders to receive mail notice:

Natural Fort Grazing Association
Ted Swanson - President
701 East County Road 76
Wellington, CO 80549

Edmund A. Leopold
7340 Cardinal Lane
Chagrin Falls, OH 44022

Folsom Grazing Association
C/O Willie Altenburg - President
570 E County Road 64
Fort Collins, CO 80524

Eagle Ridge Ranch Homeowners Assoc.
6635 County Road 730
Gunnison, CO 81230

Worthington Ranch LLC
4600 J Bar J Ranch Road
Carr, CO 80612

Miller Heritage LLC
7911 County Road 730
Gunnison, CO 81239

Platte River Power Authority
2000 E Horsetooth Road
Fort Collins, CO 80525

B Double T Ranch LLC
6400 Northaven Road
Dallas, TX 75230

T Lazy V Trust
Howard I. Holtzinger - Trustee
C/O Katherine M Bridwell
1505 Station Court
Fort Collins, CO 80521

Hinkle Ranch Properties LLC
County Road 730
5901 Deep Spring Cove
Austin, TX 78730

Michael B Keener
13611 Road 47
Torrington, WY 82240

Anthony Frank Ross
614 Bonanza Trail
Cheyenne, WY 82009

James Brandon
3463 Amador Circle
Colorado Springs, CO 80918

Invitation sent to Larimer and Gunnison County Landowners:

February 1, 2012

Dear _____

I am writing to you because Colorado Parks and Wildlife is planning a new wildlife research project that will be conducted on the Soapstone Prairie Natural Area and Meadow Springs Ranch in northeastern Larimer County. The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife.

I would like to invite you to an open house meeting to learn more about our project. Representatives from Colorado Parks and Wildlife and the U.S. Geological Survey will be available to describe the project and take questions regarding the proposed research as related to the environmental assessment process. Details of the meeting are found in the enclosed flyer.

I hope to have the chance to meet you on February 15, 2012 from 4 p.m. to 7 p.m. at the Leeper Center in Wellington.

Sincerely,

Dan Tripp

Wildlife Disease Researcher

Colorado Parks and Wildlife

February 1, 2012

Dear _____

I am writing to you because Colorado Parks and Wildlife is planning a new wildlife research project that will be conducted on the Miller Ranch State Wildlife Area in Gunnison County. The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife.

I would like to invite you to an open house meeting to learn more about our project. Representatives from Colorado Parks and Wildlife and the U.S. Geological Survey will be available to describe the project and take questions regarding the proposed research related to the environmental assessment process. Details of the meeting are found in the enclosed flyer.

I hope to have the chance to meet you on February 16, 2012 from 4 p.m. to 7 p.m. at the Aspinall-Wilson Conference Center at Western State College in Gunnison.

Sincerely,

Dan Tripp

Wildlife Disease Researcher

Colorado Parks and Wildlife

Notice in the North Forty News: January 30 to February 15, 2012



OPEN HOUSE NOTICE

Colorado Parks and Wildlife, together with the U.S. Geological Survey, will be hosting an open house in Wellington on Feb. 15 to provide information on a wildlife research project that will be conducted on the Soapstone Prairie Natural Area and Meadow Springs Ranch in northeastern Larimer County. The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife.

Representatives from Colorado Parks and Wildlife and USGS will be available to describe the project to the public and take questions regarding the proposed research project and the environmental assessment process.

Location: Leeper Center conference room at 3800 Wilson Ave. Wellington, CO
Date: February 15, 2012
Time: 4 p.m. to 7 p.m.
Format: Open House
Contact: Dan Tripp (970) 472-4478 or dan.tripp@state.co.us

Notice in the Gunnison Country Times: February 2 and 9, 2012

Parks and Wildlife to test vaccine locally

Colorado Parks and Wildlife (CPW), together with the U.S. Geological Survey (USGS), will be hosting an open house in Gunnison on Feb. 16 to provide information about a wildlife research project that will be conducted on the Miller Ranch State Wildlife Area.

The one-month project is a field trial of a new vaccine designed to prevent sylvatic plague in wildlife. Representatives from CPW and USGS will be available to describe the project to the public and take questions. The open house will be at the Aspinall-Wilson Center, 909 E. Escalante Dr., in Gunnison on Feb. 16 from 4 p.m. to 7 p.m.

Environmental Assessment Open House Meeting

Leeper Center, Wellington, CO

February 15, 2012

| Name | Address | Phone Number |
|------------------------|-----------------------------|--------------|
| Ray Kemp | 3036 Environmental Dr FC. | 222-0597 |
| Debra Fuggs | 215 N Mason FC | 416-2814 |
| Henry Maddux | 1344 view Lakewood | 303-236-4251 |
| Paul Marinari | P.O. Box 190 Wellington, CO | 970-980-7144 |
| Charles Rogers | Rawhide Energy Station | 970-229-5216 |
| Nancy Howard | 317 W. Prospect FC 80526 | 970-217-1471 |
| Julie Trip | 1112 Norwien Ct FC 80525 | 970-391-9486 |
| Sean Streich | 1300 W Stuart | 719-651-4194 |
| TRAVIS & APRIL LIVIERI | 3694 Mt. Oury | 568-1143 |
| Isabella Gucci Jones | " | 720-432-0415 |

Environmental Assessment Open House Meeting

Aspinall-Wilson Conference Center, Gunnison, CO

February 16, 2012

| Name | Address | Phone Number |
|----------------|---|----------------|
| Jan Tipp | 317 prospect Road Fort Collins CO 80526 | 970 472-4478 |
| Darren Long | 650 S. 11th Street Gunnison 81230 | (970) 642-4952 |
| Will Shoemaker | | 641-1414 |
| Alathan Seward | 300 W. New York Ave. Gunnison, CO | 641-7882 |
| Jim Cochran | 200 E. Virginia, Gunnison | 641-7604 |
| DAW ZADRA | 300 W. NEW YORK GUNNISON | 641-7887 |
| TERRI WEBER | P.O. Box 668, Gunnison | 641-4543 |
| GREG ZOBWICK | PO Box 1002 Gunnison | 641-3940 |
| J Wennum | 300 W. New York Gunnison | 81230 641-7060 |
| P. Ham | Western State College | 973-2094 |

Appendix B

Agency Coordination

RWP 03-09-12



March 6, 2012

Eva J. Bryson
Chief, Security and Environmental Management
United States Department of the Interior
U.S. Geological Survey
Box 25046 M.S. 205
Denver Federal Center
Denver, Colorado 80225

Re: Field Tests to Assess the Safety of a Sylvatic Plague vaccine (SPV), Gunnison and Larimer Counties, Colorado (CHS #61544)

Dear Ms. Bryson,

Thank you for your correspondence dated February 28, 2012 (received by our office on March 1, 2012) regarding the subject project.

Following our review of the documentation provided, we concur that a finding of **no historic properties affected** is appropriate for the proposed project areas within Gunnison and Larimer Counties, Colorado. We have reviewed and agree to the management recommendations that stipulate that no ground disturbance or construction will occur, that all transport vehicles will remain on existing road surface during bait distribution, and that field crews will be apprised of cultural resources concerns prior to project implementation. We do request the opportunity to comment on Project Area 3 if and when treatment occurs therein.

Should unidentified archaeological resources be discovered during the course of the project, work must be interrupted until the resources have been evaluated in terms of the National Register of Historic Places eligibility criteria (36 CFR 60.4) in consultation with our office.

Thank you for the opportunity to comment. If we may be of further assistance please contact Mark Tobias, Section 106 Compliance Manager, at (303) 866-4674 or mark.tobias@state.co.us.

Sincerely,


for Edward C. Nichols
State Historic Preservation Officer
ECN/MAT

WWW.HISTORYCOLORADO.ORG

HISTORY COLORADO CENTER 1200 BROADWAY DENVER COLORADO 80203

RCVD 02-29-12



United States Department of the Interior

FISH AND WILDLIFE SERVICE
Ecological Services
764 Horizon Drive, Building B
Grand Junction, Colorado 81506-3946

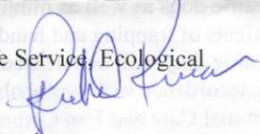


IN REPLY REFER TO:
ES/CO:USGS
TAILS 06E24100-2012-I-0108

February 27, 2012

Memorandum

To: Chief, Security and Environmental Management, U.S. Geological Survey, Denver, Colorado

From: Acting Western Colorado Supervisor, Fish and Wildlife Service, Ecological Services, Grand Junction, Colorado 

Subject: Field Studies to Assess the Safety of Sylvatic Plague Vaccine

This memorandum responds to your February 1, 2012, letter requesting concurrence with your determination that U.S. Geological Survey (USGS) activities associated with the proposed field studies to assess the safety of sylvatic plague vaccine may affect, but is not likely to adversely affect mountain plover (*Charadrius montanus*), Gunnison's prairie dog (*Cynomys gunnisoni*), Gunnison sage-grouse (*Centrocercus minimus*), and greater sage-grouse (*Centrocercus urophasianus*). We recently withdrew our proposal to list the mountain plover as threatened under the Endangered Species Act (ESA) of 1973, as amended. As a result, the mountain plover has no status under the ESA and consultation pursuant to section 7 of the ESA is not required for this species. Gunnison's prairie dog, Gunnison sage-grouse, and greater sage-grouse are all candidates for listing under the ESA and we appreciate you considering them in your project planning.

Other species assessed for project impacts included black-footed ferret (*Mustela nigripes*), Preble's meadow jumping mouse (*Zapus hudsonius preblei*), Canada lynx (*Lynx canadensis*), North American wolverine (*Gulo gulo luscus*), whooping crane (*Grus americana*), least tern (*Sterna antillarum*), Mexican spotted owl (*Strix occidentalis lucida*), piping plover (*Charadrius melodus*), yellow-billed cuckoo (*Coccyzus americanus*), *Phacelia formosula* (North Park phacelia), *Spiranthes diluvialis* (Ute ladies'-tresses), *Gaura neomexicana* spp. *coloradensis* (Colorado butterfly plant), *Spiranthes diluvialis* (Ute ladies'-tresses orchid), *Platanthera praeclara* (Western prairie fringed orchid), *Physaria congesta* (Dudley Bluffs bladderpod), *Physaria obcordata* (Dudley Bluffs twinpod), *Penstemon scariosus* var. *albifluvis* (White River beardtongue), and *Astragalus microcymbus* (skiff milkvetch). You have determined that the project will not effect these species due to species absence and/or lack of suitable habitat in the project areas, thus consultation for them is not required. Therefore, these species will not be addressed further in this consultation.

REV 04-20-09

The project will be carried out in prairie dog colonies at three locations: Project Area 1 is located in Gunnison County, Colorado, approximately 10 miles north of the town of Gunnison; Project Area 2 is located in Larimer County, Colorado, approximately 30 miles north of Fort Collins; Project Area 3 is located in the Bureau of Land Management (BLM) Wolf Creek management area in southern Moffat County and northern Rio Blanco County, Colorado. The proposed action consists of spreading oral plague vaccine (OPV) near active prairie dog burrows in each of the three project area locations. The OPV-laden bait is capable of conferring immunity to prairie dogs after ingestion. The OPV has been shown to be safe in numerous animals, including prairie dogs, black-footed ferrets, and other species. Prior experimental studies have shown no adverse effects, morbidity, or mortality resulting from the use of OPV on prairie dogs. The project will also include the trapping and visual assessment of prairie dogs and other non-target small mammals using live traps and monitoring activity at prairie dog burrows.

The project includes conservation measures that address the trapping and handling of Gunnison's prairie dogs as well as minimize potential disturbance to Gunnison's and greater sage-grouse. Effects of trapping and handling Gunnison's prairie dogs will be insignificant and all Gunnison's prairie dogs will be released unharmed. Trapping and handling of prairie dogs will be conducted in accordance with protocols and guidelines approved by the Colorado Parks and Wildlife, Animal Care and Use Committee. The project will begin after June 15, thus will avoid the critical lekking and peak nesting periods for Gunnison and greater sage-grouse. Field crews will be trained to identify and avoid the nests of Gunnison and greater sage-grouse. In addition, all work will be conducted on foot to minimize noise and potential vegetation disturbance.

Based on the proposed conservation measures, we concur that the proposed project will not adversely impact Gunnison's prairie dogs, Gunnison sage-grouse, and greater sage-grouse, nor will the project jeopardize the continued existence of these species.

Based on the information you have provided, no further consultation or conference pursuant to section 7 of the ESA is required at this time. If you become aware of new information regarding the design of the proposed project, or of impacts observed but not considered/addressed or the occurrence of other listed species within the project areas, please contact us as soon as possible for potential reinitiation of section 7 consultation or conference.

If you have any questions, or if the Fish and Wildlife Service can be of further assistance, please contact Dan Reinkensmeyer at the letterhead address or (970) 243-2778, extension 39.

DReinkensmeyer:USGSFieldStudiesAssessSylvaticPlagueVaccineCL.docx:022712:KM

Appendix C

Compliance with Environmental Statutes

From the *US Geological Survey Manual* (2002) <http://www.usgs.gov/usgs-manual/handbook/hb/445-1-h/ch1.html>

Chapter 1 Authority, Purpose, and General Policies:

1. Scope: This Handbook established the US Geological Survey (USGS or Bureau) policy for compliance with both statutory and regulatory requirements and the management of USGS environmental programs.

A. Applicability.

- (1) This manual applies to all USGS facilities and organizations.
- (2) The major Federal environmental statutes contain waivers for sovereign immunity that require USGS facilities to comply not only with Federal, but also State and local substantive and procedural requirements. Applicable Federal, State, and local requirements or Executive Orders (EO) which are more stringent than this Handbook will be followed.
- (3) State and local regulatory programs may establish regulations which are more stringent than the Federal requirements. Each USGS facility should obtain copies of its respective State and local regulations to determine if the facility is subject to requirements that go beyond the Federal laws and regulations.

The following table lists some of the Federal legal mandates that are pertinent to the proposed action. This list is representative, not exhaustive, and is compiled for information, not for legal purposes.

Pertinent Federal Legal Mandates – representative, not exhaustive

| Element | Authority | Compliance |
|--|---|---|
| Air Quality | The Clean Air Act of 1970, as amended (42 USC 7401 et seq.) National Emissions Standards for Hazardous Air Pollutants (40 CFR Parts 61 and 63) | Proposed action does not require air quality permitting. |
| Bald Eagles | Bald Eagle Protection Act (16 USC 668). | Response from USFWS analysis found that no endangered or threatened species are known to occupy the project area. (2-27-2012). |
| Cultural, Archeological and Historical Resources | National Historic Preservation Act, as amended (16 USC 470); Antiquities Act of 1906 (16 USC 431-433); Archeological and Historic Preservation Act (AHPA) of 1974 (16 USC 469 et seq.); Archaeological Resources Protection Act of 1979 (16 USC 470(aa) et seq.); Historic Sites, Buildings and Antiquities Act of 1935 (16 USC 461-462, 424-467; 49 Stat.666), as amended National Register of Historic Places (36 CFR 60) Protection of Historic and Cultural Properties (35 CFR 700) | Correspondence with the CO SHPO concerning Cultural Resource Assessment Section 106 Review (3-6-2012) states: “... <i>no historic properties affected. We do request the opportunity to comment on Project Area 3 if and when treatment occurs therein.</i> ” |

| | | |
|--------------------------|---|---|
| Endangered Species | Endangered Species Act of 1973 (16 USC 1531 et seq.) | Correspondence from the USFWS (2-27-2012) notes <i>“Based on the proposed conservation measures, we concur that the proposed project will not adversely impact Gunnison’s prairie dogs, Gunnison sage-grouse, and Greater sage-grouse, nor will the project jeopardize the continued existence of these species.”</i> |
| Energy | Energy Policy Act (EPACT) of 2005 (PL 109-58) National Energy Conservation Policy Act of 1978 (PL 95-619) EO 12759, April 15, 1991, Federal Energy Management EO 12902, March 8, 1994, Energy Efficiency and Water Conservation at Federal Facilities EO 13123, June 3, 1999, Greening the Government Through Energy Efficient Management | Proposed action does not impact energy resources nor does it produce greenhouse gases. |
| Environmental Justice | EO 12898, February 11, 1994, Environmental Justice | Proposed action does not impact minority or low-income populations inequitably. |
| Environmental Protection | National Environmental Policy Act (NEPA) of 1969 as amended (PL 91-190, 42 USC 4321 et seq.) | The proposed action is in compliance with all requirements and regulations. |
| Farmland | Farmland Protection Policy Act (7 U.S.C. 4201, et seq.) | Proposed action will not convert farmland to nonagricultural use. |
| Floodplains | Watershed Protection and Flood Prevention Act (16 U.S.C. 1101, et seq. 33 U.S.C. 701b) EO 11988, May 24, 1977, Floodplain Management Floodplain Management (42 CFR 26951) | Proposed action does not impact national or local waterways and does not require construction of flood protection measures.. |

Pertinent Federal Legal Mandates – representative, not exhaustive

| Element | Authority | Compliance |
|---------------------------|--|--|
| Hazardous and Solid Waste | Hazardous and Solid Waste Amendments of 1984 (PL 98-616) Federal Facilities Compliance Act of 1992 (PL 102-386) Hazardous Materials Transportation Uniform Safety Act of 1990 (PL 101-615) Pollution Prevention Act of 1990 (42 USC 13101 et seq.) Resource Conservation and Recovery Act of 1976, as amended (42 USC 2901 et seq.) Toxic Substances Control Act of 1976 (15 USC 2601 et seq.) Solid Waste Disposal Act of 1965, as amended (42 USC 3251 et seq.) EO 12856, August 3, 1993, Federal Compliance with Right-to-Know Laws and Pollution Prevention Requirements EO 12873, October 20, 1993, Federal Acquisition, Recycling and Waste Prevention EO 13101, September 15, 1998, Greening the Government Through Waste Prevention, Recycling, and Federal Acquisition | Bait ingredients are food grade, FDA approved and do not contain any hazardous substances. Uneaten baits will be removed from the ground at the end of the study period. |
| Health and Safety | Occupational Safety and Health Act of 1970 (29 USC 651 et seq) Occupational Safety and Health Standards (29 CFR 1910) | All actions proposed will comply with appropriate health and safety regulations and standards. |

Pertinent Federal Legal Mandates – representative, not exhaustive

| Element | Authority | Compliance |
|-----------------|--|---|
| Migratory Birds | Migratory Bird Treaty Act of 1918, as amended, 16 USC 703-71 | Response from USFWS analysis found that no endangered or threatened species are known to |
| Noise | Noise Control Act 1972 (42 U.S.C. Sec 4901 et seq.) | All bait distribution will be conducted on foot and transport vehicles will use and remain on established roads. |
| Noxious Weeds | Federal Noxious Weed Act of 1974 (7 USC 2801 et seq.) Noxious Plant Control Act of 1968 (45 USC 1241 et seq.) Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990 (16 USC 4701, 104 Stat. 4761, Title I of P.L. 101-646) EO 13112, February 3, 1999, Invasive Species Carlson-Foley Act of 1968 (PL 90-583) | The proposed action will not distribute seeds and plants and bait distribution will be conducted on foot to further reduce unintentional transport of seeds. Personnel will be trained to avoid infested areas. |
| Soil | Soil Conservation Act of 1938 (16 USC 5901 et seq.) | The proposed action will not disturb the soil and bait distribution will not chemically alter the soil composition. |
| Water Quality | Clean Water Act of 1977, as amended, (PL 95-217, 33 U.S.C. 1251 et seq.) – Section 401 Oil Pollution Act of 1990 (PL 101-380, 33 USC 2701 et seq.) Pollution Prevention Act of 1990 (42 USC 13101 et seq.) Water Quality Act of 1965 (PL 89-234) Safe Drinking Water Act (SDWA) of 1974 (42 USC 3000(f) et seq.) | The proposed action will have no impacts to surface or ground water. |

Pertinent Federal Legal Mandates – representative, not exhaustive

| Element | Authority | Compliance |
|----------|---|--|
| Wetlands | Section 404 (USC 1344) Clean Water Act Section 401 (33 USC 1341) Clean Water Act Section 10 (33 USC. 403) Rivers and Harbor Act. North American Wetlands Conservation Act, 16 U.S.C. Sec. 4401 et seq. EO 11990, May 24, 1977, Protection of Wetlands | Any impacts to waters of the US; including but not limited to; rivers, streams, ditches, coulees, lakes, pond and their adjacent wetlands requires appropriate permitting through the US Army Corps of Engineers (6/22/2011) |
| Wildlife | Fish and Wildlife Conservation Act of 1980 (16 USC 2901 et seq) Wildlife and Fisheries (40 CFR 1-End) | No additional permits or actions are required for implementation of the proposed project |

Notes:

CFR – Code of Federal Regulations

EO – Executive Order

PL – Public Law

Stat. – Statute

USC – United States Code

Appendix D

Published Information About Prairie Dogs and Sylvatic Plague



Protecting Black-Footed Ferrets and Prairie Dogs Against Sylvatic Plague

Scientists at the USGS National Wildlife Health Center (NWHC), in collaboration with colleagues at other federal agencies and the University of Wisconsin, are developing and testing vaccines that can be used to protect black-footed ferrets and prairie dogs against plague. The black-footed ferret is commonly regarded as the most endangered mammal in North America, and sylvatic plague is a major impediment to its recovery. The three prairie dog species (Gunnison's, black-tailed, and white-tailed prairie dogs), upon which the ferret depends for food and whose burrows they use for shelter, have been drastically reduced from historical levels, resulting in the near extinction of the ferret. All three species are considered 'at risk' and have been petitioned for listing as 'threatened' or 'endangered' by the U.S. Fish and Wildlife Service (FWS). Additionally, the Utah prairie dog is listed as threatened and the Mexican prairie dog is considered endangered in Mexico. Like the black-footed ferret, all five prairie dog species are highly susceptible to plague and regularly experience outbreaks with devastating losses. Controlling plague outbreaks is a vital concern for ongoing recovery programs and conservation efforts for both prairie dogs and ferrets.



Utah prairie dog (NWHC).

Sylvatic Plague – A Continued Threat to North American Wildlife

Sylvatic plague, caused by *Yersinia pestis*, is a bacterial disease transmitted by fleas that afflicts many mammalian species, including humans. For many species of wildlife, plague mortality is a serious conservation issue. In fact, more than half of North American rodent species considered of conservation concern by the International Union for Conservation of Nature reside within the range of plague outbreaks in the western U.S. The bacterium that causes plague was inadvertently introduced into North America in the early 1900's. Because it is foreign to the evolutionary history of North American mammals, most species have little or no immunity and succumb quickly to the disease. Prairie dogs are particularly susceptible to plague and suffer high mortality rates (90% or more) during outbreaks, often resulting in local or even regional extinctions. Black-footed ferrets are also highly susceptible to plague, contracting the disease by ingestion of infected prey or via bites from infected fleas. But even if they manage to avoid plague exposure, prairie dog population declines that result from a severe outbreak are devastating for ferret populations as they rely almost exclusively on prairie dogs for their survival. Plague is now considered endemic throughout the western states, but was newly discovered in the Conata Basin, South Dakota, in May 2008. This site is the most successful black-footed ferret reintroduction site in the U.S. and was considered plague-free until this outbreak.

The Black-Footed Ferret, Once Thought Extinct, Returns to the Wild

The black-footed ferret once occurred across a large mid-continent area from southern Canada to northern Mexico. The ferret depends almost exclusively on prairie dogs for food and for shelter in their burrows. Over the past century, prairie dog populations, and ferrets by extension, have drastically declined due to habitat loss, poisoning, and devastating outbreaks of sylvatic plague. Prairie dogs numbers have been reduced by over 95%.



Historical range of the black-footed ferret.

Once presumed to be extinct, a wild population of ferrets was discovered in 1981 in Wyoming. Unfortunately, this last colony succumbed to disease, but not before it provided a few animals to start a captive breeding effort that to date has produced over 7,000 young. Six facilities now maintain separate, intensively managed, captive ferret populations totaling around 290 animals.

Since 1991, over 2,600 ferrets have been reintroduced into the wild across the western U.S. and Mexico, and Canada. Ferret numbers in the wild total about 1,000 individuals as of fall 2010, with perhaps half surviving to breed each spring. Although highly successful so far, the ferret recovery program will not be complete until larger numbers of ferrets exist in the wild and their populations are sustainable.

One of the biggest obstacles to fulfilling this goal is sylvatic plague, a disease highly lethal to both prairie dogs and ferrets. In May 2008, plague was discovered at Conata Basin, South Dakota for the first time, where the largest breeding population of black-footed ferrets resided. Nearly two-thirds of the prairie dogs at Conata Basin died from plague along with about one-third (100) of the ferrets. Efforts to control the outbreak included pesticide application, and trapping and vaccination of ferrets, but these are costly, labor-intensive, short-term solutions. Consequently, continued research into the development of oral vaccines to prevent plague in prairie dogs is needed.



U.S. Fish and Wildlife Service

Successful Immunization of Black-Footed Ferrets

Scientists at NWHC have demonstrated in the laboratory that vaccination can protect black-footed ferrets from plague.



USGS scientist injects a vaccine into a black-footed ferret (NWHC).

Collaborative field studies with the FWS and the USGS Fort Collins Science Center have verified that vaccination can improve ferret survival in the wild. The plague vaccine used for ferrets is an injectable protein, developed for humans by the U.S. Army Medical Research Institute for Infectious Disease. To provide full immunity against plague, two injections of the vaccine, spaced about 30 days apart, are required. Since 2008, all captive-born ferrets released into the wild as part of the recovery program receive two doses of plague vaccine prior to release. This vaccine was also used to protect wild-born ferrets during the plague outbreak in the Conata Basin. However, field vaccination is difficult because of the time-consuming tasks of finding, capturing, handling and injecting free-ranging animals. Unfortunately, immunization of ferrets against plague will not prevent the potential loss of their prey base – prairie dogs. Ultimately, successful management of plague in ferrets will depend on managing the disease in prairie dogs.

Vaccine-Laden Bait: A New Method for Field Application

Current efforts to halt the spread of plague in prairie dog colonies typically rely on dusting individual prairie dog burrows with



FWS scientist sprays pesticide on a prairie dog burrow to kill plague-carrying fleas (Randy Matchett, FWS).



A variety of vaccine-laden oral baits (NWHC).

pesticides that kill plague-infected fleas. However, this pesticide application is labor intensive, costly and difficult to sustain over time. As an alternative approach, scientists at NWHC, in collaboration with the University of Wisconsin–Madison, have developed a plague vaccine for prairie dogs that can be delivered via oral bait. Laboratory studies have shown that voluntary consumption of this vaccine-laden bait by different prairie dog species results in significant protection against plague infection. Work has now shifted to optimizing baits and distribution methods for field delivery of the vaccine. Peanut butter-flavored baits were shown to be preferred by prairie dogs in laboratory studies, and preliminary field studies using baits without vaccine have shown rates of uptake by wild prairie

dogs > 90% within 3-4 days of application. The vaccine remains viable within the baits for up to 7 days at 28° C. Additional work is in progress or planned to test dispersal of baits from planes or vehicles and to further confirm the safety of the vaccine in non-target animals, such as other rodents and domestic animals. The ultimate goal is to license vaccine-laden bait for field application in targeted areas where black-footed ferrets are living or are released as part of the recovery program. It could also be used in national parks or urban areas where the potential for human exposure to plague-infected rodents is high. Using vaccine-laden bait provides effective immunity against plague that would allow treatment of more prairie dogs, with less labor and expense than dusting. Better protection of prairie dogs would minimize the risk of disease transfer to ferrets, aid in prairie dog conservation and protect public health. Developing effective vaccines for ferrets and prairie dogs will enhance recovery of the endangered black-footed ferret.



Black-tailed prairie dog in New Mexico (J. Chipault, NWHC).

Oral Plague Vaccine (OPV) Work Group Established

Under the direction of the Executive Committee of the Black-footed Ferret Recovery Implementation Team, a work group was established in December 2010 to complete development and delivery of the OPV as a management tool to combat plague in prairie dogs and promote the recovery of the black-footed ferret. As part of the strong interagency foundation of the Work Group, the Western Association of Fish and Wildlife Agencies will play a vital role in overseeing the OPV project. The OPV Work Group will draw from the broad expertise and experience of governmental (federal, state, and tribal) and nongovernmental partners to promote and coordinate the use of OPV for the conservation and recovery of black-footed ferrets and prairie dogs in targeted locations.

For additional information, please contact:

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U.S. Fish and Wildlife Service



Sylvatic Plague Vaccine Frequently Asked Questions

PLAGUE

Q: What is plague?

A: Plague is a disease caused by bacteria called *Yersinia pestis*. It is transmitted by fleas and afflicts many kinds of mammals, including humans. In the United States, 10-20 people are diagnosed with plague each year. The disease is treatable with antibiotics.

Q: What are the symptoms of plague?

A: Symptoms usually start 2-6 days after becoming infected. Symptoms include fever, chills, weakness, and swollen and painful lymph nodes. The infection can spread from the lymph nodes to other parts of the body, including the lungs. There are three types of plague: bubonic (infection of the lymph nodes), septicemic (infection of the blood), and pneumonic (infection of the lungs). Pneumonic plague can be spread from person-to-person and must be treated immediately to prevent death.

Q: How do you contract plague?

A: Most often, people become infected by being bitten by infected fleas. People may also become infected by handling infected animals or their tissues. People can also become infected by breathing in the bacteria, such as from other people or animals with pneumonic plague who are coughing.

Q: Where does plague occur in the U.S.?

A: Most human cases in the United States occur in two regions: 1) northern New Mexico, northern Arizona, and southern Colorado; and 2) California, southern Oregon, and far western Nevada.

Q: Why should I be worried about plague in wildlife?

A: Wild animals, especially rodents, can act as a source of plague for people and their pets. Rock squirrels and their fleas are the most frequent sources of human infection in the southwestern states. For the Pacific states, the California ground squirrel and its fleas are the most common source. Many other rodent species, for instance, prairie dogs, wood rats, chipmunks, and other ground squirrels, suffer plague outbreaks and some of these occasionally serve as sources of human infection. Dogs and cats that roam the outdoors may bring infected fleas back to the home that can then bite people. Pets, especially cats, can become infected by hunting and eating infected rodents and pass the infection on to people. People who handle

infected wild animals may become infected through flea bites, contamination of open wounds, or inhaling the bacteria.

Q: What does plague do to prairie dogs?

A: Prairie dogs are highly susceptible to plague and regularly experience outbreaks with devastating losses; 90% or more of the prairie dogs in a colony can die during an outbreak, often resulting in local or even regional extinctions. Plague mortality is a serious conservation issue for prairie dogs and the animals that depend on them.

Q: Why should we care about prairie dog population management?

A: Some prairie dog species in Colorado are candidates for listing as threatened or endangered species by the U.S. Fish and Wildlife Service. Successful development of a plague vaccine will provide a tool to manage prairie dog populations in some areas so further listing action will be unnecessary.

Q: Why is prairie dogs an important part of the ecosystem?

A: Prairie dogs play a key role in the prairie and montane ecosystems. Numerous kinds of animals depend on prairie dogs and their colonies for food and habitat. Burrowing owls and mountain plovers depend on prairie dog colonies for nesting and breeding habitat. Prairie dog burrows are used as homes by many animals, including burrowing owls, rabbits, badgers, weasels, and snakes. Other animals, such as the swift fox, coyote, weasels, hawks, eagles, and the endangered black-footed ferret rely on prairie dogs for food. Although the vegetation around prairie dog colonies can be sparse, it is more nutritious than plants on uncolonized areas, because the digging activities of prairie dogs help to aerate the soil allowing greater water penetration, and their dung acts as a fertilizer.

SAFETY AND PREVENTION

Q: How can I tell if an animal has plague?

A: Often the first sign of a plague outbreak in prairie dogs and other rodents is a noticeable absence of the animals where they had previously been plentiful. Dead rodents may be found with blood oozing from their nostrils or mouth. Pet cats that are infected can become severely ill very quickly with high fever and swollen lymph nodes in the neck. Prompt veterinary treatment is important.

Q: What can I do to reduce the risk of plague infection for me and my pets?

A: It is important to avoid contact with wild rodents and their fleas. Do not pick up or touch dead animals. If plague has recently been found in your area, report any observations of sick or dead animals to the local health department or law enforcement officials. Eliminate sources of food and nesting places for rodents around homes, work places, and recreation areas; remove brush, rock piles, junk, cluttered firewood, and potential food supplies, such as pet and wild animal food. Make your home rodent-proof. If you anticipate being exposed to rodent fleas, apply insect repellent to clothing and skin, according to label instructions, to prevent flea bites. Wear gloves when handling potentially infected animals. If you live in areas where rodent

plague occurs, treat pet dogs and cats for flea control regularly and do not allow these animals to roam freely. Health authorities may use appropriate chemicals to kill fleas at selected sites during animal plague outbreaks.

SYLVATIC PLAGUE VACCINE

Q: What is sylvatic plague vaccine?

A: Sylvatic plague vaccine (SPV) is a modified raccoon poxvirus that produces two proteins of *Yersinia pestis*. These proteins do not cause plague but act to stimulate the production of antibodies against plague. The vaccine is contained in peanut butter-flavored bait that is readily eaten by prairie dogs.

Q: How does a prairie dog get vaccinated by eating this bait?

A: When a prairie dog eats the peanut butter-flavored bait containing the vaccine, the tissues inside the animal's mouth are exposed to the vaccine. The prairie dog will produce antibodies that help protect them from plague if bitten by an infected flea. In laboratory studies, consumption of baits by prairie dogs resulted in significant protection against plague infection.

Q: How long does the vaccine last?

A: Available research suggests this vaccine should be effective for at least 9 months in prairie dogs and probably longer. However, it is difficult to determine how immune systems in individual animals will respond to the vaccine.

Q: How do you know which animals eat the bait?

A: The baits contain a biomarker, Rhodamine B, providing a safe, non-lethal way to determine if an animal has eaten the bait. After an animal eats the bait, the biomarker is incorporated into hair and whiskers as they grow. Whiskers of animals that have eaten baits luminesce when viewed under a special microscope. Biomarker is also excreted in the feces which turn a red color.

Q: Is this bait dangerous?

A: No. The vaccine in these baits cannot cause plague and has been shown to be safe in several kinds of animals. Other vaccines that use the same virus have been shown to be safe in numerous types of animals, including rabbits, sheep, cats, and dogs.

Q: Can I get plague from contact with the vaccine?

A: No. The vaccine does not contain whole plague bacteria, but only two genes from the bacterium. The virus that carries these genes has been altered to reduce its ability to cause disease.

Q: What if I find SPV-laden bait?

A: It is best to leave the bait where you found it. The risk of human infection with the vaccine virus is low, but it is best to avoid contact. If you need to pick up the bait, wear gloves or use a plastic bag to avoid skin contact with the bait. As with any biological matter, wash your hands thoroughly with soap and water after any contact with bait.

Q: What if my dog or cat eats SPV-laden bait?

A: The virus used in the vaccine has been shown to be safe in many different kinds of animals, including domestic dogs and cats. Eating a large number of baits may cause a temporary upset stomach in your pet but does not pose a long-term health risk. Do not attempt to remove bait from your pet's mouth; doing so may cause you to be bitten. If your pet becomes ill from consuming baits, please contact your veterinarian.

Q: Can I use this bait to vaccinate my dog or cat?

A: No. This vaccine is only for use in prairie dogs. Currently, there is no plague vaccine for pets.