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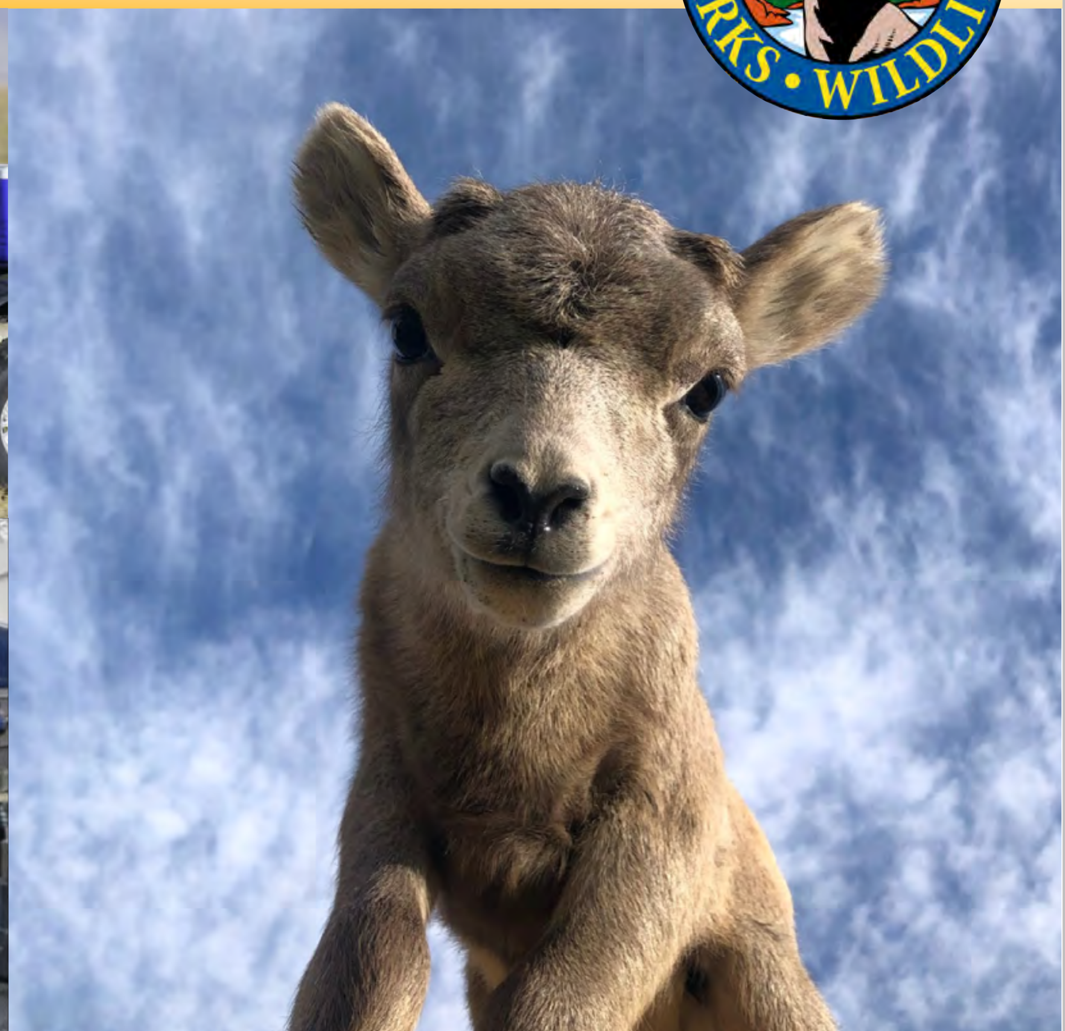
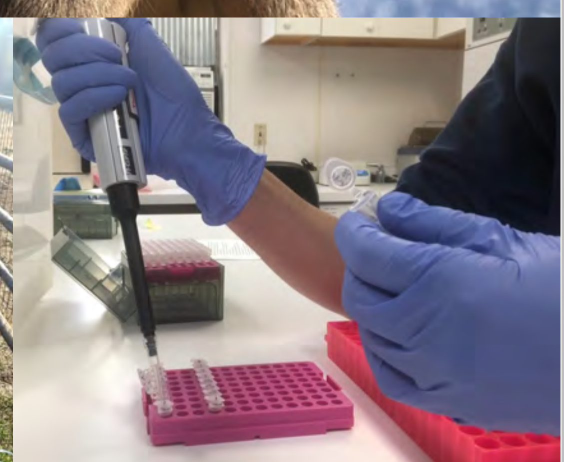
Wildlife Research Reports

Wildlife Health

July 2023—June 2024





WILDLIFE RESEARCH REPORTS

JULY 2023-JUNE 2024

WILDLIFE HEALTH PROGRAM

COLORADO PARKS AND WILDLIFE

Foothills Wildlife Research Facility, Fort Collins, CO

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EXECUTIVE SUMMARY

This wildlife research report represents summaries of wildlife research projects and other activities conducted by the wildlife health program of Colorado Parks and Wildlife (CPW) from July 2023 through June 2024. These research efforts represent both short-term and long-term projects in various stages of completion. Projects are designed to provide tools and information to benefit conservation and management of wildlife in Colorado. In addition to research, the wildlife health program provides a number of services to aid CPW in managing Colorado's wildlife.

Wildlife health research focused on diseases that affect the long-term health and viability of wildlife populations including chronic wasting disease (CWD) in cervids, respiratory disease in bighorn sheep, and plague in prairie species. Chronic wasting disease work focused heavily on optimizing CWD diagnostics for surveillance and monitoring in elk populations and evaluating management approaches in free-ranging populations. Plague research focused on working toward better understanding of the prediction and control of plague epidemics in prairie and shrub-steppe systems. Bighorn sheep respiratory disease work focused on improving understanding of respiratory pathogens and methods for managing disease. Work in these areas involved assessments of technologies that may have applications for large-scale management of these and other wildlife health problems in Colorado and elsewhere. Additional work focused on development of diagnostics and tools to support wildlife management work including disease management tools and development of tools to facilitate safe capture and collaring of wildlife.

Wildlife health management services during 2023–2024 continued to emphasize detection and monitoring of important wildlife health problems via examination of field case submissions and conducting disease diagnostics. The wildlife health program also provided training, field, and laboratory functions for many other CPW management and research programs. In addition to these more general activities, we continued to support statewide CWD surveillance efforts by facilitating sample processing, overseeing database entry and quality control, and providing sampling training, focused testing services, and targeted surveillance.

Numerous collaborators supported our work. First and foremost, are the CPW field personnel across the state who report sick or dead wildlife and collect and transport carcasses and samples to support wildlife health diagnostics and disease surveillance. Additionally, members of the general public are invaluable in their prompt reporting of sick wildlife to local field offices. Such reports are the foundation of many wildlife health investigations. We also continued working in collaboration with the Colorado Department of Public Health & Environment and the Colorado Department of Agriculture to prevent, investigate, and (as needed) control important diseases that are shared with humans and domestic animals. Additional collaborators include: the CPW Wildlife Commission, Colorado State University, City of Fort Collins, CPW big game auction-raffle grants, USGS National Wildlife Health Center, United States Department of Agriculture – Agricultural Research Services and Wildlife Services, World Wildlife Fund, Advantage Bio Consultants, Species Conservation Trust Fund, Texas A&M Veterinary Diagnostic Laboratory, Wyoming State Veterinary Laboratory, and Wyoming Game and Fish Department.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Black-footed ferret and black-tailed prairie dog population responses to plague management in Colorado

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Dan Tripp

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Plague epidemics in prairie ecosystems have contributed significantly to the overall decline of Colorado's prairie dog populations, as well as other species of wildlife that depend on prairie dogs as prey or on landscapes modified by their activity, including the endangered black-footed ferret (*Mustela nigripes*).[1] Consequently, understanding and controlling plague has emerged as a critical need for conserving imperiled prairie dog populations, black-footed ferrets and other native species of concern in Colorado.[2]

With the implementation of the Natural Resources Conservation Service (NRCS) Black-footed Ferret Initiative and the Programmatic Safe Harbor Agreement, as well as the passage of SB169 and HB 1267, Colorado has become an important location for the recovery of the black-footed ferret. Nine release sites have been identified since 2013 with ~500 ferrets released and additional sites could be identified in the near future. Unfortunately, some of these reintroduction sites were lost to plague. Management of plague at current and future black-footed ferret release sites is vital to ensure success of the reintroduction efforts.[3] Annual management to limit plague and stabilize existing black-tailed prairie dog (*Cynomys ludovicianus*) populations in northern Colorado is ongoing and necessary to sustain reintroduced black-footed ferrets at the Soapstone Natural Area and Meadow Springs Ranch complex administered by the City of Fort Collins.

The scale of plague management at selected sites in Colorado has increased as new disease management tools have been developed.[4, 5] In 2017-19, about 1,700 acres at the Soapstone Natural Area and Meadow Springs Ranch black-footed ferret reintroduction site were treated with oral vaccine and/or insecticidal dust.

Previous Colorado Parks and Wildlife (CPW) research evaluating prairie dogs' serological response to a more economical vaccine dose (0.2x) has demonstrated positive antibody responses (~26% seroconversion). However, the magnitude of this response is less than the seroconversion (>50%) observed in a study using captive prairie dogs (Cárdenas-Canales et al. 2017).

More recently, we conducted trials with captive prairie dogs to assess antibody responses to different doses of oral plague vaccine (CPW unpublished data). We evaluated bait vaccine doses of ~5.9×10^7 median tissue culture infectious dose ($TCID_{50}$, "1x"), ~1.2×10^8 $TCID_{50}$ ("2x"), and ~4.8×10^8 $TCID_{50}$ ("8x"). Antibody responses to the 1x and 2x dose baits averaged ~40% positive overall. The 8x dose baits stimulated a positive response (and stronger responses) in ~90% of vaccinated prairie dogs. These findings highlight crucial differences in vaccine titration methods used by the current commercial vaccine manufacturer and USGS, who developed the vaccine (Rocke et al. 2014). A 3-day vaccine titration method used by USGS underestimated by ~8-fold the true amount of virus nominally designated as the standard "5×10^7 pfu" vaccine dose.

In 2020, we distributed 8x baits during field trails at 50 baits/acre on black-tailed prairie dogs plots. Antibody responses to the 8x dose baits in free-ranging prairie dogs averaged ~40% seropositive overall. No adverse effects of vaccination with 8x baits were observed. These results suggest that some individuals (adult females) may consume multiple baits, depriving younger prairie dogs of the opportunity to find and consume vaccine baits. Increasing bait density is likely to boost bait uptake in juvenile prairie dogs, which is needed to provide greater population-level protection from plague. [7, 8]

Recent *in vitro* work has shown that the amount of virus detectable in cell cultures can be increased 4–8-fold by treating the vaccine solution with ethylenediaminetetraacetic acid (EDTA). The EDTA treatment breaks up large aggregates of live vaccine virus, thereby making more individual virus particles biologically available. This improved bioavailability was demonstrated *in vivo* during a trial in which ~86% of captive prairie dogs that voluntarily consumed a bait containing a 1x dose of vaccine treated with EDTA (hereafter 1x EDTA) had positive antibody responses to vaccination (CPW unpublished). No adverse effects of vaccination with 1x EDTA baits were observed. In light of these findings, further investigation of free-ranging prairie dogs' antibody responses to vaccine dosage (8x and 1x EDTA) and bait density was conducted in 2021.

In 2021, we conducted prairie dog occupancy and burrow activity surveys (Tripp et al. 2018) on 38 colonies/plots at the Soapstone Prairie and Meadow Springs Ranch complex. We distributed ~273,500 doses of vaccine in ~171,200 baits on ~2,256 acres at the Soapstone Prairie and Meadow Springs Ranch complex. We also applied experimental vaccine baits (1x EDTA and 8x dose) on ~515 acres spanning 2 research plots and captured and sampled 196 prairie dogs on these plots. On the research plots, 31-50% of the captured prairie dogs were seropositive for the V antigen (stimulated by the vaccine).

In 2022, we conducted prairie dog occupancy and burrow activity surveys (Tripp et al. 2018) on 41 colonies/plots at the Soapstone Prairie and Meadow Springs Ranch complex. We distributed ~137,500 doses of vaccine on ~1,831 acres at Soapstone Prairie Natural Area and ~74,000 doses of vaccine on ~986 acres on Meadow Springs Ranch. We also began a pilot evaluation of plague management tools (fipronil grain bait and deltamethrin dust) to control fleas on prairie dogs. We measured prairie dog and small mammal survival and abundance on treatment and control plots. We used camera traps deployed before and after fipronil grain treatment to observe if/what non-target species are attracted to fipronil grain bait.

In 2023, we continued to manage plague and evaluate flea control tools at the study area. We also began a project to monitor the diversity and abundance of arthropods before and after the application of insecticide treatments on the plots.

Study location

The study area is the Soapstone Prairie Natural Area/Meadow Springs Ranch colony complex, owned by the City of Fort Collins (Figure 1).

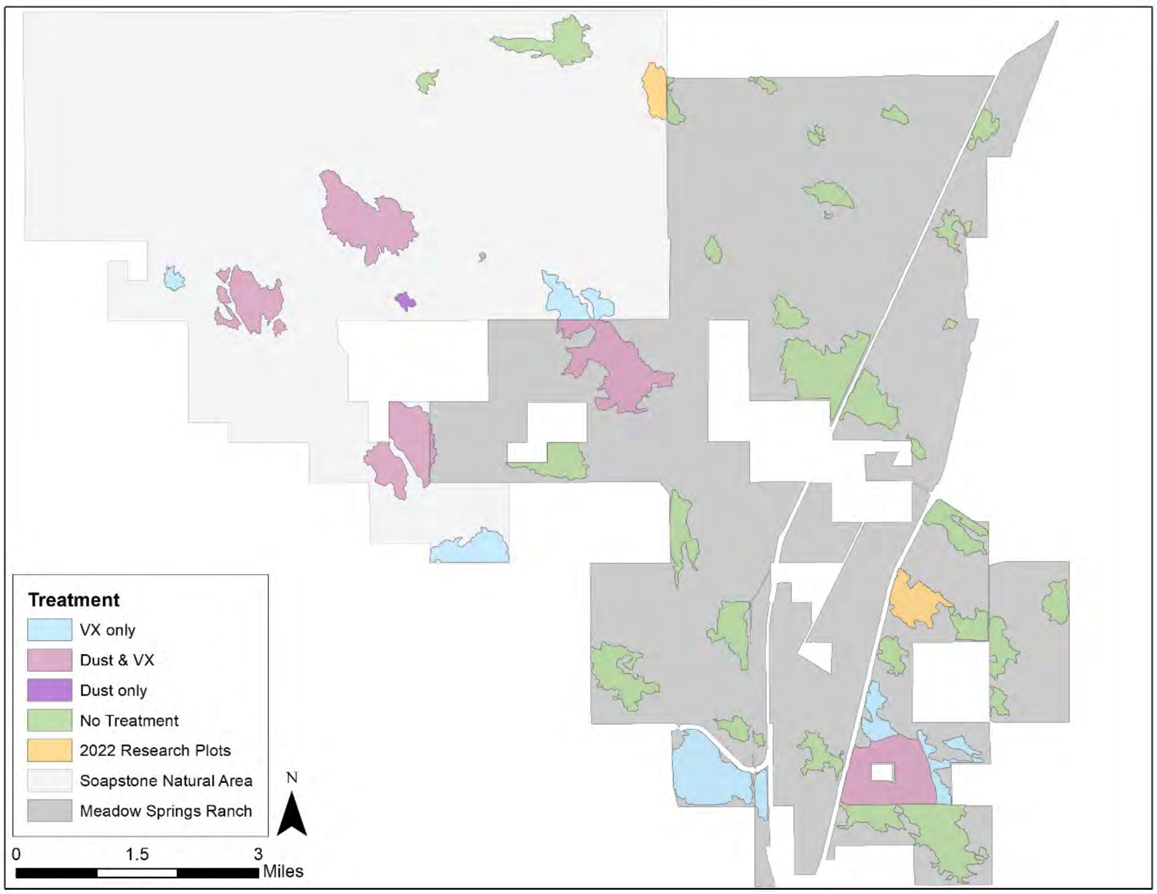

Figure 1. Occupied acres and treatment areas at the Soapstone Prairie Natural Area/Meadow Springs Ranch colony complex in 2023.

Methods

In 2023, we manufactured vaccine baits (~1 g dry weight) at CPW's Foothills Wildlife Research Center. Our modified bait recipe [9] includes ~3 parts double-distilled water, 2 parts Incortrix® bait powder (FoodSource Lure Corporation, Birmingham, Alabama), 1 part organic peanut paste, 0.01 part FD&C Blue #2 food dye, and a volume of EDTA treated finished viral fluid needed to achieve the target concentration.

Vaccination of prairie dogs occurred by voluntary ingestion of vaccine-laden baits distributed within treated prairie dog colonies. We distributed 75 baits/acre, in an attempt to increase bait uptake. We distributed baits on-foot and used ATV-mounted equipment to deliver bait along transects with 10-30 meter spacing. We conducted prairie dog occupancy and burrow activity surveys in June and September following reported methods.[4]

In 2023, 12 months after the application of fipronil grain bait and deltamethrin dust, we evaluated the degree of flea control on prairie dogs. We measured prairie dog and small mammal survival and abundance on treatment and control plots. We also began a project to monitor the diversity and abundance of arthropods before and after the application of insecticide treatments on the plots.

<u>Preliminary Results:</u>

We conducted prairie dog occupancy and burrow activity surveys on 41 colonies/plots at the Soapstone Prairie and Meadow Springs Ranch complex. [4] We distributed ~133,500 doses of vaccine on ~1,780 acres at Soapstone Prairie Natural Area and ~86,500 doses of vaccine on ~1,150 acres on Meadow Springs Ranch.

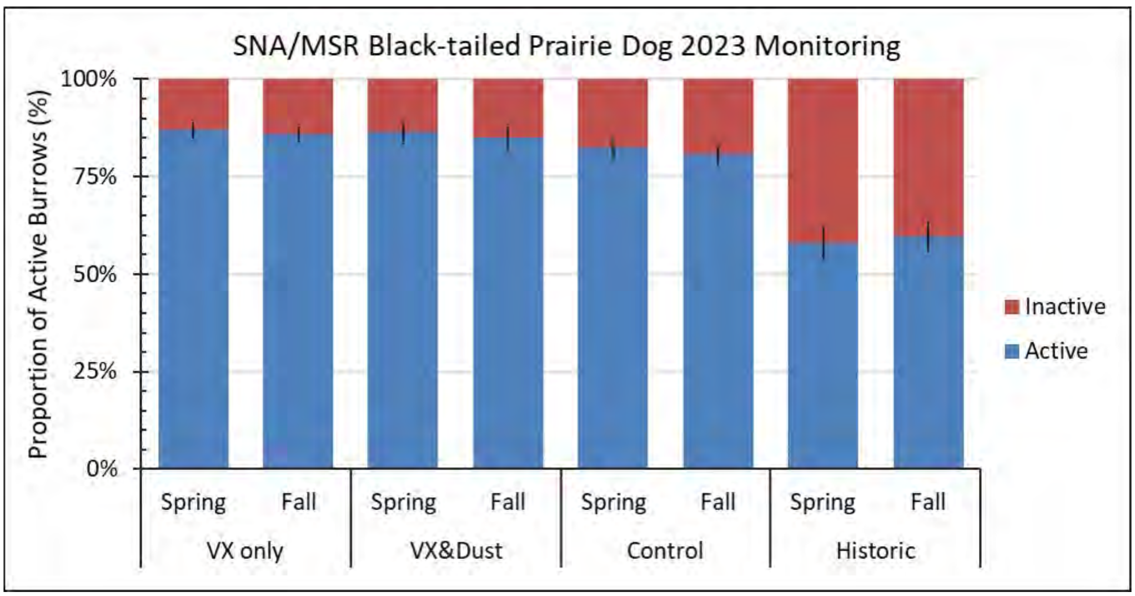

Figure 2. Proportion prairie dog burrows scored as active (blue) and inactive (red) during surveys conducted in the spring and autumn 2023 on colonies receiving plague management at the Soapstone Prairie and Meadow Springs Ranch complex. Data summary and analysis are preliminary.

We conducted prairie dog occupancy and burrow activity surveys in the spring and fall on 41 colonies/plots receiving combinations of dust, vaccine and no treatment at the Soapstone Prairie and Meadow Springs Ranch complex in 2023 (Figure 2). We will use these data to monitor responses to plague management though time. In 2023, colonies expanded on the Soapstone Prairie Natural Area from 1,895 to 2,264 acres and on Meadow Springs Ranch from 4,802 to 5,553 acres (Figure 3). Effective plague management is the likely stabilizing colonies in treated areas while persistent drought may be driving colony expansion on untreated areas (Figure 3).

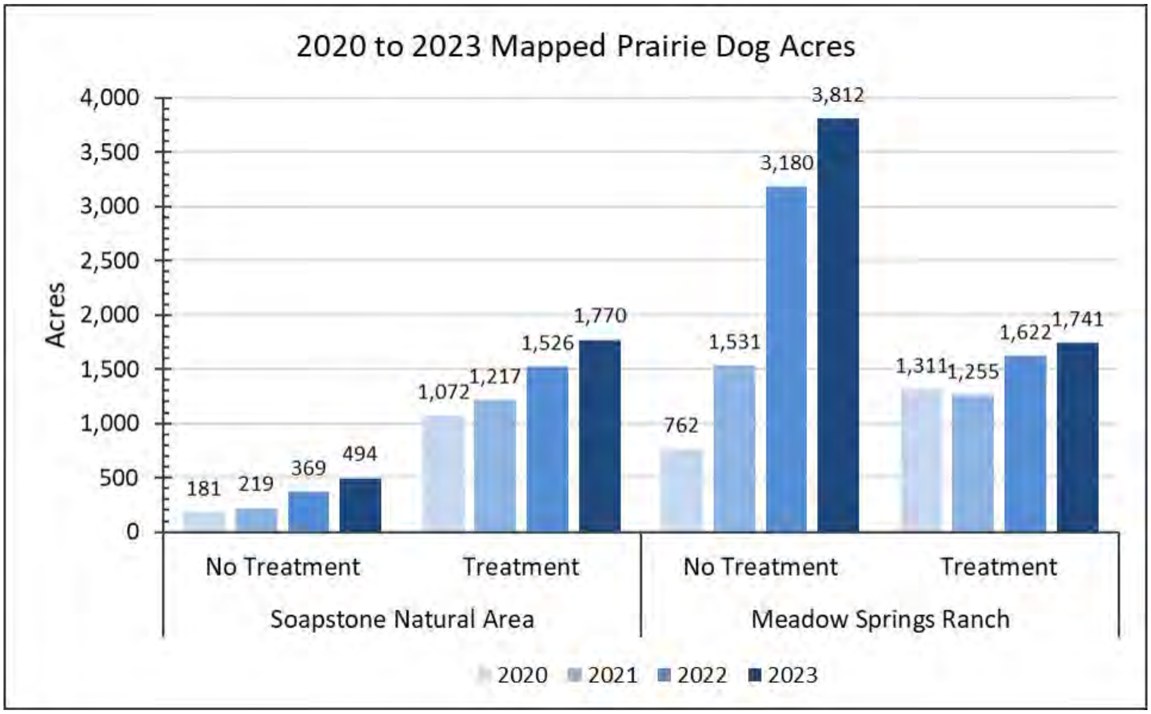

Figure 3. Area of mapped acres in 2020-2023 on non-treated ("no treatment") colonies and colonies receiving plague management ("treatment") at the Soapstone Prairie and Meadow Springs Ranch complex.

We distributed ~90 grams of fipronil grain at each prairie dog burrow on research plots and 6-7 grams of deltamethrin dust in each prairie dog burrow in August 2022. In 2023, 12 months after the application of fipronil grain bait and deltamethrin dust, we captured ~480 prairie dogs and ~92 small mammals on fipronil grain, deltamethrin dust and control plots.

Preliminary analysis indicates that ~12 months after the treatments were applied, flea abundance was ~1.5 fleas per prairie dog on insecticide treatment plots. On control plots, flea abundance was ~7.6 fleas per prairie dog. Further preliminary analysis indicates that ~12 months after the treatments were applied, flea abundance was ~1.2 fleas per small mammal on treatment plots. On control plots, flea abundance was ~4.5 fleas per small mammal. Results for abundance and survival of prairie dogs and small mammals on treatment plots are pending further analysis.

In April, 2023, we installed 480 pitfall traps on 16 sites at the Soapstone Prairie Natural Area and Meadow Springs Ranch sites. Throughout 2023, we collected arthropod abundance and species diversity data before and after the application of insecticide treatments on the plots. We captured, identified and released ~13,000 individuals of the insect orders Araneae, Coleoptera, Hemiptera, and Orthoptera.

In 2024, any remaining grant funds will be used to support the continued evaluation of plague management tools at the Soapstone Prairie and Meadow Springs Ranch complex. We will continue to analyze data collected in 2020-2023 and prepare for continued field research in 2024.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Preventative plague management and continued sylvatic plague vaccine research on soapstone prairie and meadow springs ranch

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Dan Tripp

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Plague epidemics in prairie and shrub steppe ecosystems cause dramatic reductions in prairie dog and black-footed ferret populations.[1,2] Plague has contributed significantly to the overall declines of Colorado's three prairie dog species and has negatively impacted efforts to prevent the extinction of the black-footed ferret (*Mustela nigripes*).[1,2] Consequently, understanding and controlling plague has emerged as a critical need for conserving reintroduced black-footed ferrets and the black-tailed prairie dog populations in which they are released.[3]

Beginning in 2009, the city of Fort Collins has managed plague on the Soapstone Prairie Natural Area and Meadow Springs Ranch in an effort to conserve black-tailed prairie dog populations and the habitat they create for additional species of concern. These efforts have stabilized black-tailed prairie dog populations in the managed areas to the extent that black footed ferrets were reintroduced in 2014. Currently, the city of Fort Collins manages plague on ~1,500-2,000 acres of black-tailed prairie dog colonies annually. An additional ~1,500-4,000 acres are left untreated and are at risk of collapse from plague. Furthermore, the city of Fort Collins has collaborated with Colorado Parks & Wildlife on studies to evaluate the efficacy of sylvatic plague vaccine and other plague management tools. In 2013-15, as part of a multi-state research project, Colorado Parks and Wildlife distributed experimental vaccine and placebo baits on black-tailed prairie dog colonies on the two city of Fort Collins properties.[8] Continued collaboration with the city of Fort Collins to provide plague management support for additional acres while also continuing to evaluate plague management tools in select areas will help to ensure that stable populations of black-tailed prairie dogs exist to support black footed ferrets and the numerous other wildlife species they support.[1–7]

As an extension of long-term research, we have developed practical approaches for preventing plague outbreaks in prairie dog colonies, and we have managed plague on ~1,500 to 2,900 acres of black-tailed prairie dog colonies at the Fort Collins sites (Table 1). Plague management follows protocols previously developed in conjunction with our plague management research.[9–12] We have also estimated the size of managed colonies annually, opportunistically collected samples for plague surveillance, and assessed prairie dog occupancy and activity using burrow activity as a proxy for counts (Figure 1). In addition to sustaining an annual plague management effort, we have continued to evaluate and adaptively use oral plague vaccine and flea control tools to manage plague.

Table 1. Acres of black-tailed prairie dog habitat treated with insecticidal dust by the Fort Collins Natural Area Program and oral vaccine administered by CPW in 2016-2023. Data summary and analysis are preliminary.

Year	Treatment	Acres
2016	Dust	964
	Vaccine	1,230
2017	Dust	1,039
	Vaccine	1,668
2018	Dust	1,044
	Vaccine	1,712
2019	Dust	1,217
	Vaccine	1,722
2020	Dust	1,437
	Vaccine	1,805
2021	Dust	1,500
	Vaccine	2,257
2022	Dust	1,570
	Vaccine	2,817
2023	Dust	1,780
	Vaccine	2,933

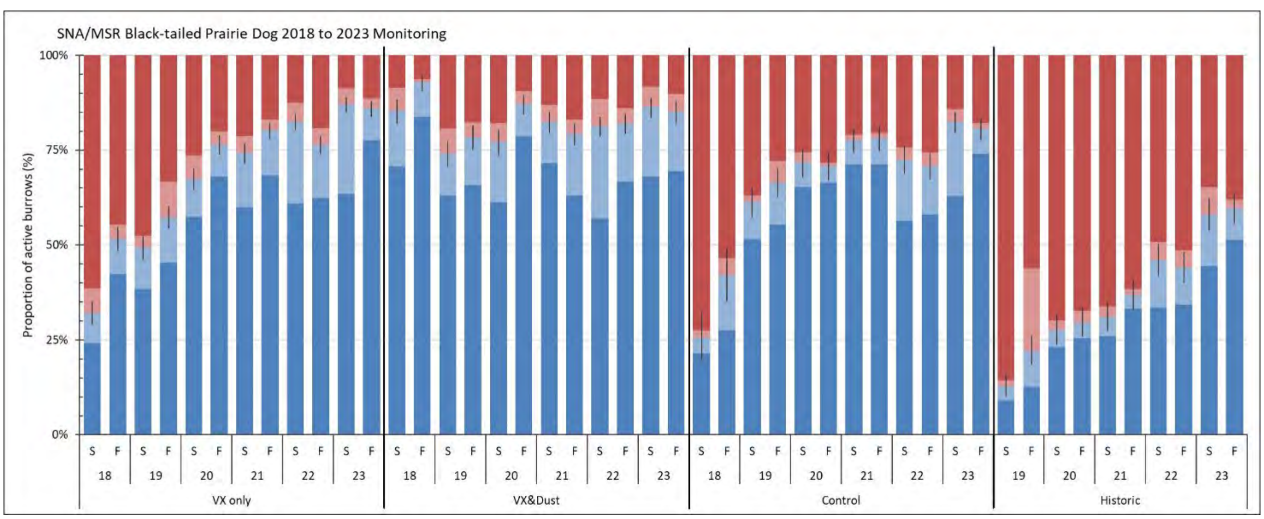

Figure 1. Proportion prairie dog burrows scored as active (blue), likely active (light blue), inactive (red) and likely inactive (light red) during surveys conducted in the spring (S) and autumn (F) 2018-2023 on colonies receiving plague management (vaccine, dust) or no treatment (control, historic) at the Soapstone Prairie and Meadow Springs Ranch complex. Data summary and analysis are preliminary.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Safety and efficacy of fipronil treated grain to control fleas in free-ranging black-tailed prairie dogs and nontarget small mammals

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Dan Tripp

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Plague epidemics in prairie and shrub steppe ecosystems have contributed significantly to the overall decline of Colorado's prairie dog populations, as well as other wildlife species that depend on prairie dogs as prey or on habitat modified by their activity. Impacted species of concern include the burrowing owl, mountain plover, ferruginous hawk, swift fox, kit fox, and the endangered black-footed ferret (*Mustela nigripes*).[1] Consequently, understanding and controlling plague has emerged as a critical need for conserving imperiled prairie dog populations, black-footed ferrets and other native species of concern in Colorado.[2]

Adaptive plague management in Colorado is ongoing and has evolved as disease management tools and strategies have been developed.[3-5] Deltamethrin dust application into burrows (hereafter: "dusting") has been shown to be effective at controlling fleas and suppressing the spread of plague throughout prairie dog colonies.[3, 4, 6] Dusting, therefore serves immediate conservation needs to prevent further population declines of these species. However, fleas may develop resistance to deltamethrin with repeated use,[7] and this tool may become less effective when applied with suboptimal equipment and/or poor application methods.[8]

Potential impacts of deltamethrin to nontarget small mammals are difficult to interpret. Dombro reported that deer mouse (*Peromyscus maniculatus*) abundance and survival was not impacted by dusting prairie dog burrows.[9] However, Goldberg reported that apparent survival of small mammals was negatively associated with deltamethrin-laced products (dust and liquid spray in bait stations, nests and burrows).[10]

Recently, a fipronil grain bait was developed to control fleas on rodents. Grain (wheat) treated with 0.005% fipronil was shown to be effective in systemically controlling fleas on prairie dogs.[11-13] There is however, conflicting evidence over the suitability of fipronil for use in integrated pest management programs.[14]

In this experiment, we applied 0.005% fipronil bait on two plots (≤100 acres each) and compared flea abundance and prevalence on prairie dogs and small mammals captured on these plots to those captured on dusted and control plots. We will also compare prairie dog and small mammal survival and abundance on treatment (fipronil bait and dust) and control plots. We also used cameras to observe if nontarget species interact with or consume fipronil bait.

Study location

We captured free-ranging prairie dogs and small mammals at field sites in Colorado where plague management programs are on-going. Study areas for black-tailed prairie dogs include plots/colonies at Soapstone Prairie and Meadow Springs Ranch located on natural areas owned by the City of Fort Collins.

Methods

We will implement a before-after-control-impact (BACI) experimental design to evaluate treatment impacts on prairie dogs and small mammals at three time points. Non-treated plots will provide baseline data before and after treatment. This design will allow us to measure and compare flea abundance and prevalence, as well as survival and abundance of prairie dogs and small mammals between treatment and control sites over the study period.

We have included two experimental replicates for black-tailed prairie dogs and small mammals. Each replicate includes: 1 plot that received fipronil grain bait at ~113 grams/burrow, 1 plot that received dust at 4-6 grams/burrow and 1 control plot that received no insecticide treatments. Replicated treatment groups were needed to inform on potential variation within and between colonies/plots and to aid in designing potential future management applications. We will assigned whole colonies or plots (≤100 acres) within larger colonies a treatment to avoid small plot bias. The capture schedule followed a robust design methodology to estimate abundance and survival.

To determine baseline flea prevalence and abundance and to mark individuals to for survival and abundance analysis, prairie dogs and small mammals were captured from the study plots prior to treatment. All captured individuals were ear-tagged or pit-tagged (Monel tag 1005-1, National Band and Tag Co, Bio-Mark Inc.). Fleas were collected from individual prairie dogs and small mammals and tested for *Y. pestis* by PCR as per ongoing study protocols.

Vector control efforts using 0.05% deltamethrin dust were conducted by the City of Fort Collins Natural Areas Dept. as part of ongoing plague management programs. Once annually, burrows were infused with a target of 4–6 g of dust.[3, 6, 8, 15] Fipronil grain bait was applied according to label instructions. We visited 50 randomly selected burrows and observed the amount of bait remaining each day for 10 days after fipronil bait distribution (Figure 1). This data helped us to understand how long fipronil bait may remain above ground (not consumed by prairie dogs) and therefore available to nontarget species.

Prairie dogs and small mammals were captured at ~30 days and 12 months post flea control and fleas were collected from individual animals as per ongoing study protocols. After sampling, animals were immediately released.

In 2022, we deployed ~30 Reconix trail cameras at 30 randomly selected prairie dog burrows on the fipronil treatment plots. Cameras were deployed for ~7 days before treatment and again for ~7 days after fipronil bait was distributed. Cameras installed 30.5 cm above the ground and 2.75 meters from the center of the burrow opening captured images of prairie dogs and small mammals interacting with and consuming bait. This design helped us understand what species may be attracted to and consume fipronil bait.

Preliminary results

In 2022, we captured and processed ~790 prairie dogs, ~180 ground squirrels and ~290 mice. We collected ~6,500 fleas from prairie dogs and other small mammals. We captured 83,400 photos in 938 "camera days". Prairie dogs were captured in ~19,000 photos while birds (primarily horned larks) were captured in ~2,000 photos.

In 2023, we captured and processed ~481 prairie dogs, ~205 ground squirrels and ~196 mice. We collected ~1,970 fleas from prairie dogs and other small mammals. Flea treatments appeared to suppress fleas on prairie dogs captured ~30 days and 12 months post treatment (Figure 2).

Future work

In 2024, we will conduct prairie dog capture at 24 months post treatment. Flea abundance and prairie dog abundance and survival will be measured and compared to data collect before and 30 days and 12 months rafter treatment.

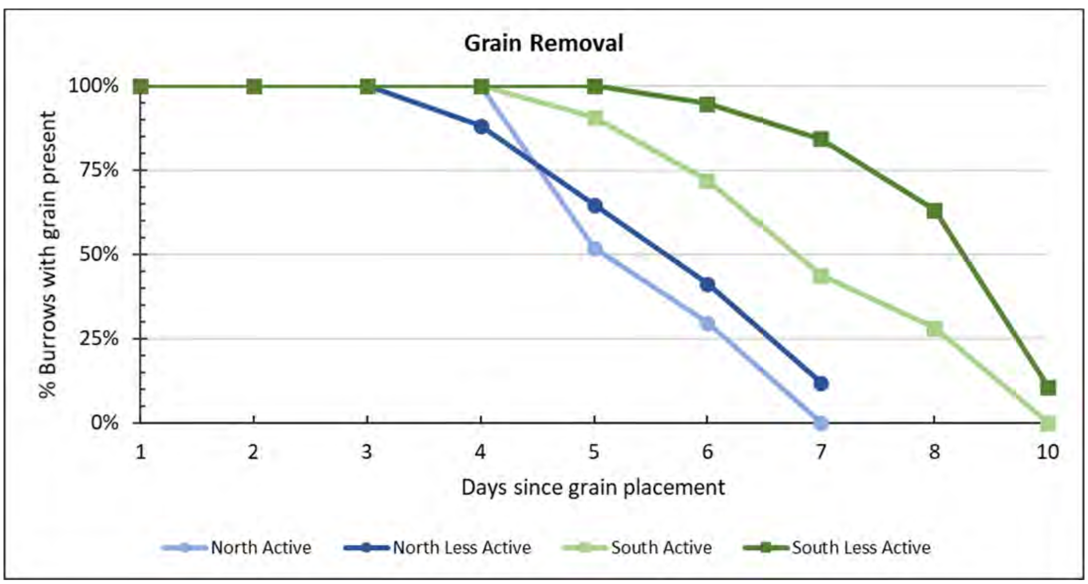

Figure 1. Fipronil grain removal from active and less active prairie dog burrows in the 10 days after application on North and South treatment plots in 2022. Data summary and analysis are preliminary.

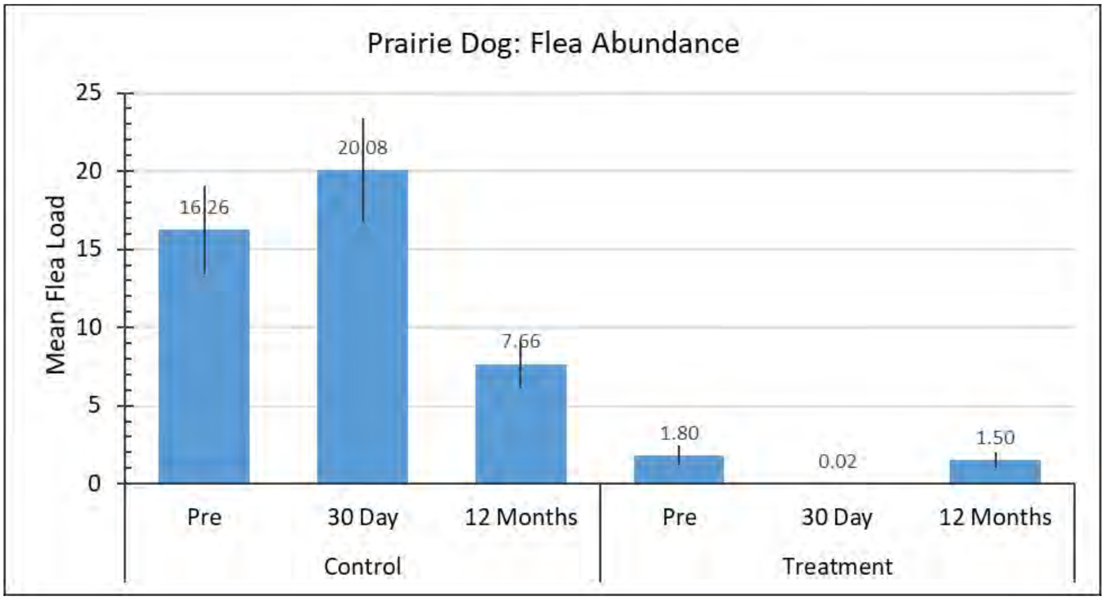

Figure 2. Flea abundance on prairie dogs before, ~30 days after and 12 months after flea control treatments on treatment and control plots in 2022-2023. Data summary and analysis are preliminary.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Impacts of insecticides used in plague management programs on non-target arthropods

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Dan Tripp

Plague epidemics in prairie and shrub steppe ecosystems have contributed significantly to the overall decline of Colorado's prairie dog populations, as well as other wildlife species that depend on prairie dogs as prey or on habitat modified by their activity. Impacted species of concern include the burrowing owl, mountain plover, ferruginous hawk, swift fox, kit fox, and the endangered black-footed ferret (*Mustela nigripes*).[1] Consequently, understanding and controlling plague has emerged as a critical need for conserving imperiled prairie dog populations, black-footed ferrets and other native species of concern in Colorado.[2]

Adaptive plague management in Colorado is ongoing and has evolved as disease management tools and strategies have been developed.[3-5] In 2022, about 6,000 acres of occupied black-tailed prairie dog (*Cynomys ludovicianus*) habitat that support black-footed ferret recovery in Colorado were treated with oral *Yersinia pestis* Vaccine, deltamethrin dust or fipronil grain bait.

Deltamethrin dust application into burrows (hereafter: "dusting") has been shown to be effective at controlling fleas and suppressing the spread of plague throughout prairie dog colonies.[3, 4, 6] However, fleas may develop resistance to deltamethrin with repeated use,[7] and this tool may become less effective when applied with suboptimal equipment and/or poor application methods.[8]

Oral baits infused with insecticide can systemically control ectoparasites that feed on rodents.[9-11] Fipronil is an insecticide that has been shown to control several arthropod species during field trials. Recently, a fipronil grain bait was developed to control fleas on rodents. Grain (wheat) treated with 0.005% fipronil was shown to be effective in systemically controlling fleas on prairie dogs.[10-13] There is however, conflicting evidence over the suitability of systemic fipronil for use in integrated pest management programs.[14]

Arthropods play important roles in prairie ecosystems and can be found in greater abundance and diversity on active colonies than on neighboring grasslands.[15, 16] Although vector control has been successful at limiting plague transmission and increasing survival of ferrets and prairie dogs, little is known about the secondary effects of these treatments on nontarget arthropods.[16, 17] When used for plague control in prairie dog colonies, deltamethrin may reduce the abundance of several families of arthropods (Hymenoptera, Coleoptera, Heteroptera, Araneane).[18,]

[19] However, Dombro reported minimal impact to arthropod abundance on dusted plots.[16] Perhaps, deltamethrin delivery methods and high reproductive rates for arthropods counter any potential negative treatment effect on survival.

Further research is needed to determine what impact vector control, conducted as part of operational adaptive plague management programs, may have on nontarget arthropods in Colorado. Small mammals such as deer mice (*Peromyscus maniculatus*) and grasshopper mice (*Onychomys leucogaster*) rely on arthropods as a food source, particularly on prairie dog colonies where beetles can be particularly abundant.[20, 21] Because small mammals act as first level predators for arthropods, their reproduction and survival can be impacted by a decline in arthropod food sources.[22]

In 2023, we monitored and will compare arthropod abundance and species diversity on prairie dog colonies treated with deltamethrin dust, fipronil grain and control areas. This design will provide data to compare abundance and species diversity of arthropods on plots receiving two types of flea control and plots receiving no treatment. We will use pitfall traps to measure arthropod abundance and diversity on treatment and control plots (Figure 1).

At the Soapstone Prairie Natural Area and Meadow Springs Ranch black-footed ferret reintroduction complex we established arthropod sampling transects on 3 fipronil grain treatment plots, 6 deltamethrin dust treatment plots and 8 control plots (Figure 2). Plague management is ongoing at this site with ~10 years of deltamethrin dust treatment in some areas. Two fipronil grain treatment plots were established in 2022 with another treated in 2023. The long history of plague management at this site will allow for analysis of the potential impacts of long-term plague management on arthropod abundance and diversity.

The Greeley Natural Area, Missile Site Park, is a new study area. After a site survey this site was partitioned into 2 fipronil grain and 2 deltamethrin dust treatment areas with 4 adjacent control areas (Figure 3). With no history of plague management at this site, we will measure arthropod abundance and diversity both before and after insecticide treatments are conducted. This experimental design will allow for a before-after-control-impact (BACI) analysis of the potential impacts of the initial treatments on arthropod abundance and diversity.

We will use pitfall traps to measure arthropod abundance and diversity on treatment and control plots. On each plot we will install 30 live pit fall traps. Arthropod pitfall sampling will occur on each plot for 4 consecutive days in May-Sept. We will record the number of each group captured/counted using the most specific taxonomic group possible (Family, Genus or Species; Table 1). Voucher and unknown specimens will be collected for species identification using appropriate taxonomic keys in the laboratory.

Preliminary results and future work

In April, 2023, we installed 480 pitfall traps on 16 sites at the Soapstone Prairie Natural Area and Meadow Springs Ranch sites (Figure 2). We also installed 240 pitfall traps on 8 sites at the Greeley Natural Area site (Figure 3). Throughout 2023, we collected arthropod abundance (Figure 1) and species diversity data before and after the application of insecticide treatments on the plots. We encountered >12,500 arthropods at the Soapstone Prairie Natural Area and Meadow Springs Ranch sites and >10,500 arthropods at the Greeley Natural Area site. In 2024, we will expand the ongoing arthropod abundance and diversity project to include pollinators and dung beetles. We will design and construct pollinator pan traps and adapt existing pitfall traps to monitor dung beetle populations on sites receiving plague management.

Table 1. Order or family of arthropods expected in pitfall traps on prairie dog colonies at our study areas.

Araneae	**Coleoptera**	**Hemiptera**	**Hymenoptera**	**Other**
THOM - crab spiders	TENEB - darkling beetles	LYGAE - seed bugs	APID - bees	LEPID - butterflies
THER - widow spiders	CARAB - ground beetles	REDUV - assassin bugs	VESP - wasps	LARV - larvae
LYCO - wolf spiders	SCARAB - june/dung beetles	COREI - leaf-footed bugs	FORM - ants	DIPT - flies
SALT - jumping spiders	CERAM - long-horned beetles	PENT - stink bugs	MUTIL - velvet ants	DERMA - earwigs
GNAPH - hunting spiders	SILPH - carrion beetles	APHI - aphids		NEURO - lacewings
DYSD - woodlouse spiders	MELOD - blister beetles	CICA - leafhopper	**Orthoptera**	ISOP - termites
PHOL - cellar spiders	CINC - tiger beetles	CYDN - burrower bug	ACRID - grasshoppers	COLL - springtails
SOLP - sunspiders	COCC - ladybird beetles		GRYLL - field crickets	ACAR - mites
	CHRYS - leaf beetles		GRYAC - sand crickets	
	CURCU - weevils			

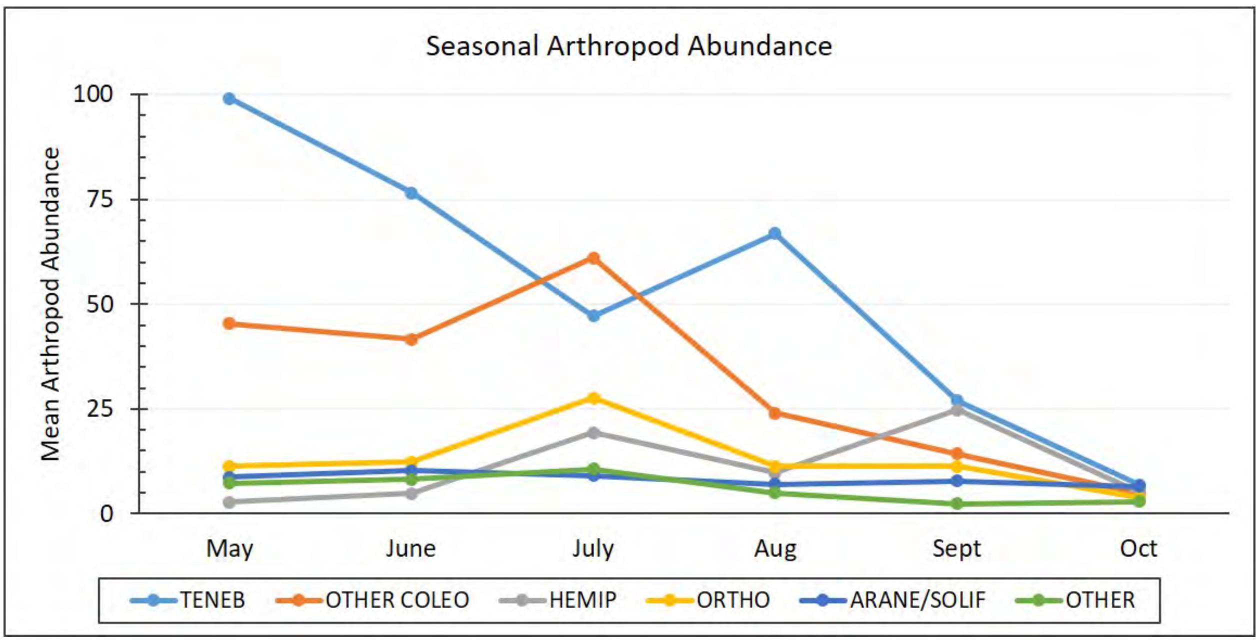

Figure 1. Seasonal arthropod abundance (Tenebrionidae, other Coleoptera, Hemiptera, Orthoptera, Araneane and other arthropods) on arthropod sampling transects at the Soapstone Prairie Natural Area and Meadow Springs Ranch field sites. Data summary and analysis are preliminary.

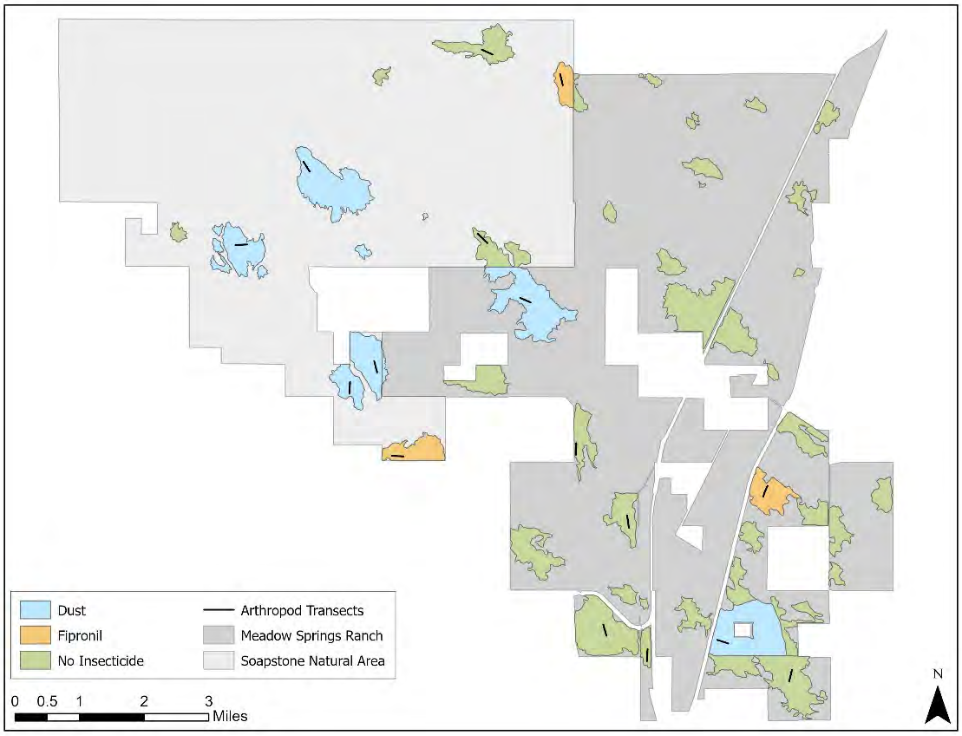

Figure 2. Treatment areas and arthropod sampling transects at the Soapstone Prairie Natural Area/Meadow Springs Ranch colony complex in 2023.

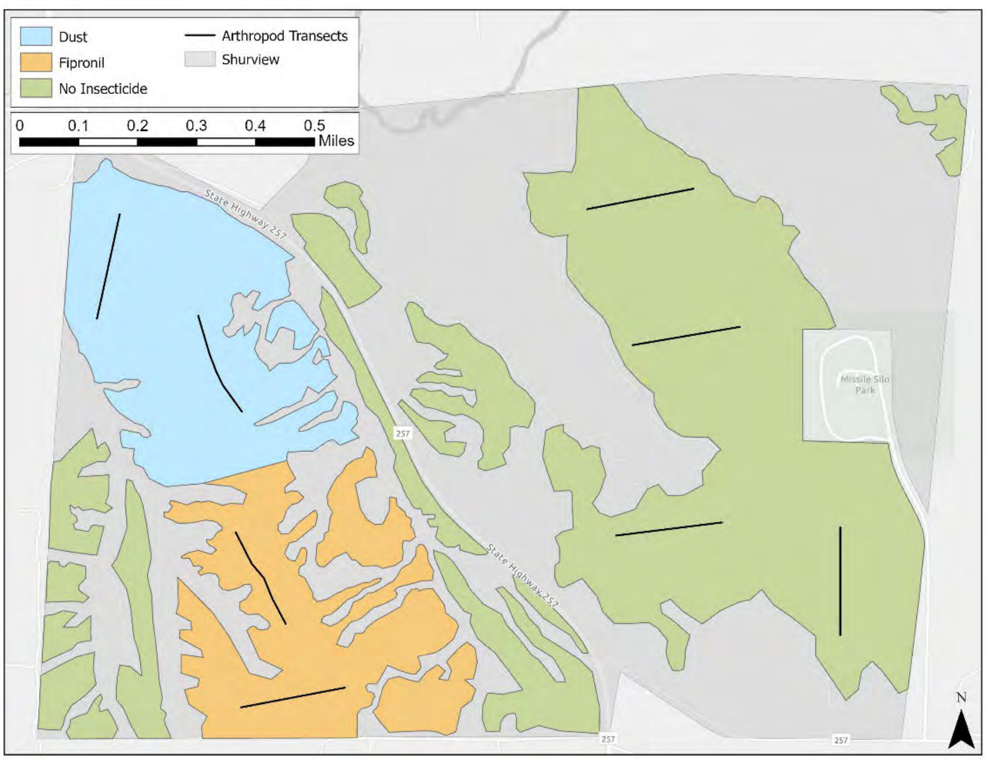

Figure 3. Treatment areas and arthropod sampling transects at the Greeley Natural Area, Missile Site Park in 2023.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Improvement of mechanical bait distribution and plague management equipment

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Dan Tripp

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Plague epidemics in prairie and shrub steppe ecosystems have contributed significantly to the overall declines of Colorado's three prairie dog species, as well as other species of wildlife that depend on prairie dogs as prey or on landscapes modified by their activity, including species of concern such as burrowing owl, mountain plover, ferruginous hawk, swift and kit fox, black-footed ferret, and perhaps Gunnison's sage grouse.[1–6] Consequently, understanding and controlling plague has emerged as a critical need for conserving imperiled prairie dog species[5] and other native species of concern in Colorado.

Plague management and research programs in Colorado have played a significant role in the US Fish & Wildlife Service's decision to refrain from federal listing of the Gunnison's prairie dog[7] and were instrumental in the white-tailed prairie dog[8] non-listing decision. Annual management to limit plague and stabilize existing prairie dog populations in western Colorado will be needed to sustain the "not warranted" listing status for both species. Additionally, management of plague at current and future black-footed ferret release sites is vital to ensure success of the reintroduction efforts.

Previous CPW collaboration with multiple partners has aided the development of vaccine bait distribution equipment for use on All-Terrain Vehicles (ATV).[9] Bait distribution with this equipment is 10-15 times more efficient than distribution on-foot. However, this equipment is obsolete, requires frequent maintenance and is often in need of repair limiting efficiency of field operations. Improved (redesigned) bait distribution equipment that is more robust and reliable than the current model is a critical need to gain efficiency and reduce the cost of plague management.

In 2023, the CPW Wildlife Health Program collaborated with the Colorado State University, Rapid Prototyping Laboratory to design and build a new bait distribution system to conduct efficient distribution of plague management baits from ATV's. This Generation 1.0

prototype device (Figure 1) successfully delivered ~94,000 baits on 1,250 acres at a rate of 91 acres/hour. This device demonstrated several improvements over pervious designs including:

- Modular and serviceable design
- Designed for operator safety and to withstand field use
- Fast and visible bait sorting mechanism
- Decreased frequency of jammed baits
- Removable bait hopper
- Eliminated the need for external batteries

Figure 1. The Colorado State University, Rapid Prototyping Laboratory Generation 1.0 prototype bait distribution system.

In 2024, we will again work with the CSU Rapid Prototyping Laboratory to further improve this design and build a generation 2.0 prototype device (Figure 2). This device will build upon the successes of the previous design while also incorporating additional improvements including:

- Design for manufacturing (DFM) to reduce future manufacturing costs
- Shorten overall height of device
- Redesign quick connects for added strength and resistance to shearing

- Design bait hopper to withstand vibration and impacts
- Motor on/off switch relocation to operator side of unit
- Main power on/off switch added to unit
- Anomaly mode (retracted plungers via additional switch/button)
- Design PCB layout to prevent power surges
- Change color of pellet backdrop to white for added visualization

In 2024, the CSU Rapid Prototyping Laboratory will produce a generation 2.0 prototype device for field testing in 2024. The above improvements and the Design for Manufacturing (DFM) process will allow CPW to evaluate this design to provide future safe, reliable, efficient and cost effective plague management equipment.

Figure 2. The Colorado State University, Rapid Prototyping Laboratory Generation 2.0 bait distribution system design.

Also in 2023, we field tested a new G-R Mfg. design for HPA backpack dusters [10, 11, 12]. These dusters utilize fire department grade self-contained breathing apparatus (SCBA) packs (Figure. 2). These SCBA dusters were easy to use and maintain with fewer valves and attachments than the previous HPA dusters. Lager air tanks eliminate the need for frequent and time consuming refilling. However, the larger tanks and SCBA packs increase the total weight of the unit which limits the time users can comfortably use this equipment. In 2024, we will pursue this design with modifications that eliminate excess weight and increase user comfort.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Development of naturally degrading wildlife collar drop-off mechanisms

Period Covered: July 1 2023 - June 30 2024

Principal Investigators: Ian Smith, Mary Wood, Jack Grider, Mat Alldredge, Pauline Nol, Eli Burns, Caleb Hollingsworth, Adam Lujan

Wild ungulates are captured and collared to support a wide array of research and management operations in Colorado. To minimize the length of time that an animal is wearing a collar, researchers utilize various drop-off mechanisms to ensure that a collar comes off an animal once data collection is complete. While electronic remote collar drop-off mechanisms are currently available; they often come with a substantial cost and may be prone to failure. Naturally degradable materials such as cotton spacers have been utilized; however, timing of drop-off is highly inconsistent and these materials often last many years beyond the battery life of the collar.

We worked with CSU engineering and materials science students to develop a simple drop-off design utilizing the degradable polymer PolyCaproLactone (PCL). This polymer is used in many medical applications for its ability to degrade in a predictable manner and can be tailored to fail within a specific period. PCL is a 3D printable material, allowing for rapid prototyping, easy manufacturing, and the ability to manufacture the device in-house if necessary. Preliminary evaluation involved tensile testing of devices with an array of different thicknesses for baseline tensile strength. Devices were placed into environmental chambers to simulate ambient conditions in Colorado. After various periods of time in the environmental chambers, devices were removed for tensile testing. Preliminary laboratory data supported a steady rate of degradation of the device.[1]

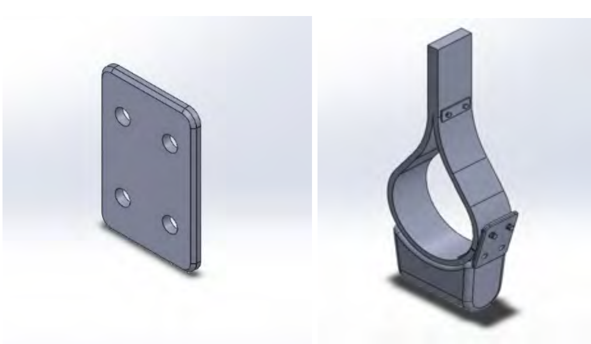

Figure 1: Initial design schematics of a PCL spacer and a typical collar with a PCL spacer. The spacers are 6 cm by 4 cm and will range in thicknesses from 0.6 mm – 1.6 mm. They will weigh ~10g. Spacers are designed to fit GPS collars just like the currently used cotton spacers, negating the need to redesign collars or change hardware.

The next stage of this work aims to more accurately model this degradation rate. Specifically our goals are to: (1) provide examples of spacer widths that last for a minimum time period, coinciding with common study lengths (i.e. 2 to 3 years) on captive wildlife; (2) determine the variance between thickness of a PCL spacer and time to natural degradation/collar failure for wildlife collars; (3) make recommendations for the appropriate width PCL spacer for typical field study time periods. To accomplish these goals we will test PCL spacers on captive wildlife collars so that we can easily observe drop-off time. The Wildlife Health Program has started this work with just three animals wearing PCL drop-off collars (e.g. Figure 2), and have an approved study plan to collar ten more animals in the coming year.

Figure 2: A collar with PCL drop-off placed on study animal NE17 on 6/14/2022. This collar dropped off naturally on 4/6/2024. The PCL spacer broke right down the center line, not near the bolts. This may have been partly due to the bend produced by this shape of collar, sometimes called a "tear-drop" shape. We do not expect to use tear-drop shaped collars for the remainder of the study.

Captive bighorn sheep and elk at the FWRF will be fitted with wildlife collars commonly used by researchers and biologists. We will use three Advanced Telemetry Systems G2110E Iridium collars designed for bighorn sheep, two Vectronic Aerospace Vertex Plus collars designed for sheep, and 5 more Vectronic Aerospace Vertex Plus collars designed for elk. All collars will be attached using a PCL spacer of varying thicknesses at a point in the collar designed for a drop-off mechanism. We will choose 10 animals for this study based on their age and propensity for normal health and behavior. These study animals will be randomly assigned to five different groups of starting thickness ranging from 0.8mm to 1.6mm (Table 2). If a collar drops in the first few months, the animal may be collared again to test the thinnest spacer (0.6mm, 6th group of spacers). So each animal may be collared up to two times during the study. A possible scenario is outlined in Table 2. Spacers will be attached to collars using a torque wrench to insure bolts are tightened equally on all collars. The date, spacer thickness, and animal ID will all be tabulated and technicians working at the facility will monitor the presence of these collars as part of their daily routine. The date of collar drop-off will be recorded and the collar will be recovered to confirm that the PCL spacer broke. The primary independent variable is the starting thickness of a PCL spacer attached to these collars. Additional factors we can account for when modeling the drop off rate include species, age of species, and weather over course of study period. We will record the date when collars naturally drop-off animals.

We will use Cox proportional-hazards models to determine the probability of a PCL spacer failing at a given point in time as a function of PCL thickness, individual activity level, species, and a random effect of individual. The Cox proportional-hazards model has two underlying assumptions: the survival function is exponentially distributed and the hazard ratio remains constant between groups throughout the study. To ensure the latter assumption is met, we will use a log minus log plot, which applies a log-log transformation to the survival function and looks to see if the plotted curves remain parallel over time. We will assess collinearity of predictor variable using a Pearson's correlations, and not include variables whose Pearson's $|r| \geq 0.70$ in the same model. We will conclude the model failed to detect an effect of a predictor variable if its 95% confidence interval includes zero. To select the best fitting model, we will remove the least influential non-significant predictor variable/variables from the full model until a minimum AICc value is reached. We will construct models within package survival in program R.[4]

Collars equipped with activity sensors will be activated and set to record activity data in 5 minute intervals; data are recorded 4 times per second and averaged.[2] This is a common setting for studies of large mammals when using collar products from Vectronic Aerospace.[3] Activity level of each study animal will be totaled, and totals will serve as an index for collar wear. We will obtain activity totals from wild elk and bighorn sheep from CPW biologists and researchers or from peer reviewed studies found online. We will conduct t-tests to compare activity levels between our captive activity totals and wild activity totals; we expect that our captive totals will be lower than wild totals. If there is a significant difference between these two groups, we will use the model built on our captive animals to extrapolate PCL spacer widths fit for wild animals and a range of study periods, allowing us to develop a set of PCL spacer recommendations for wild elk and bighorn sheep.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Pilot investigation of physiologic biologgers to measure nutritional condition of elk

Period Covered: July 1 2023 - June 30 2024

Principal Investigators: Mary Wood, Mark Ditmer, Nathaniel Rayl, Eric Bergman, George Wittemeyer, Pauline Nol

All information in this report is preliminary and subject to further evaluation. Information MAY NOT BE PUBLISHED OR QUOTED without permission of the author. Manipulation of these data beyond that contained in this report is discouraged. By providing this summary, CPW does not intend to waive its rights under the Colorado Open Records Act, including CPW's right to maintain the confidentiality of ongoing research projects. CRS § 24-72-204.

Physiological biologgers provide the capability of collecting fine-scale physiological data from free-roaming wildlife that can provide insights which behavioral studies alone cannot[1]. In combination with high frequency GPS locations, frequent physiological data provides numerous opportunities to link space use, environmental conditions[1-2], and anthropogenic disturbances[3] with internal states. Reveal LINQ Generation 3 biologgers, made by Medtronic, were developed for human use, but have been successfully and safely deployed in a variety of species including ungulates[2,4]. These devices use some of the latest medical technology to collect an array of physiological data within miniaturized housing (1.2 cc; 4.0 mm × 7.2 mm × 44.8 mm; 2.4 g; **Figure 1**[5,6]. Importantly, the devices can collect data continuously for ~ 3 years without need for a new battery. The software, specifically developed for high temporal resolution data collection in wildlife, can collect 2-minute average heart rates (bpm), several activity and posture metrics (15-minute intervals), and subcutaneous temperature (every 4 hours).

Figure 1: Left: Reveal LINQ Generation 3 biologger made by Medtronic. **Right:** Implantation procedure and location of device in a mule deer.

The latest generation of Reveal LINQ biologgers can measure bioimpedance (every 4 hours) using conductance between two nodes in the unit. Body condition metrics, including fat thickness, are typically collected infrequently because of the need to re-capture individuals. However, collecting body condition metrics are critical as they often correlate with survival and reproductive success. If the bioimpedance metric, collected 6 times a day by the biologgers can provide a similar level of inference into an individual's body condition, changes in fat thickness in GPS collared individuals can be assessed through space and time with unprecedented frequency.

Bioimpedance data (measured as conductance) collected from free-roaming mule deer in Utah have a strong negative correlation (r = - 0.7) with green vegetation availability (NDVI) based on GPS locations from the associated individuals during the winter months (**Figure 2**). These preliminary results suggest lower conductance from body fat as green forage becomes less available in the northern mountains of Utah. Bioimpedance values collected over an entire year (from a single individual) highlight variation throughout the year, with elevated values (less impedance/higher conductance) occurring during months of lactation (**Figure 2**).

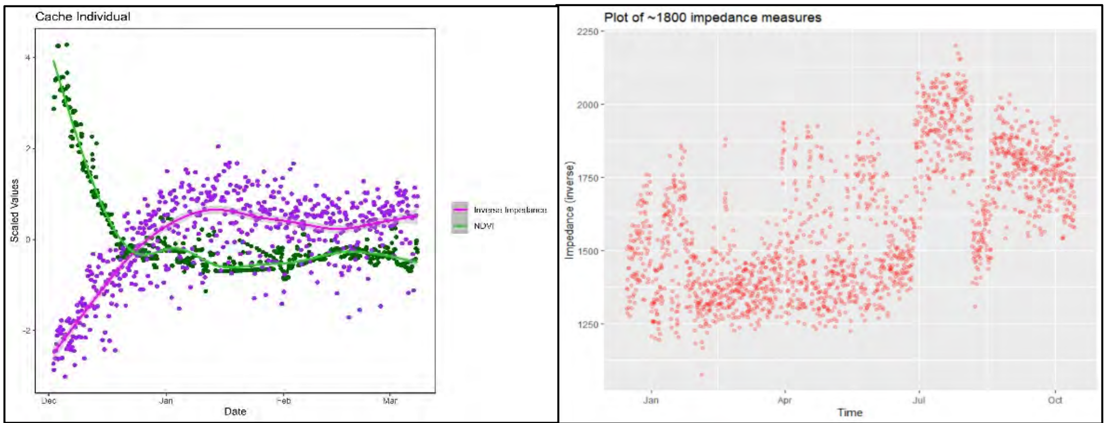

Figure 2: Left: NDVI (green) measured at the deer's GPS location in relation to inverse bioimpedance (conductance values; purple) during the winter months in Utah. **Right:** Bioimpedance values (based on conductance) for one year from a mule deer in southern Utah.

While these initial correlations are striking, understanding how well the biologgers' bioimpedance measures compare with traditional body condition metrics is critical for interpretation and informing management actions. Additionally, if the bioimpedance measure is sensitive to food availability, a variety of questions can be tested by linking GPS locations with bioimpedance estimates. For example, identifying areas of the landscape that are calorically and energetically beneficial or not would greatly enhance management of migration routes and stopover sites.

We initiated a study to determine how well high-frequency bioimpedance values register changes in body condition elicited through multi-month dietary shifts. An experimental approach with biologger-implanted captive elk during diet-controlled periods will allow us to associate biologger metrics and changes in body condition when reduced dietary allowances put elk in a net negative energy balance and when they are in stable biophysical condition and a net positive energy balance. The impact of short term, severe dietary alterations will also be assessed to see if biologger metrics identify short term changes akin to pulses (potential range:1-10 days) in diet quality or pulses in dietary constraint. This information can serve to validate the relationship between forage availability/caloric density, biologger-collected bioimpedance, and body condition scores.

Five adult female elk housed at the Foothills Wildlife Research Facility (FWRF) had biologgers implanted subcutaneously using aseptic techniques above their shoulder or in their rump. Throughout the study period, elk were fed a measured diet to mimic changes experienced by free ranging elk with abundant nutrition provided during the summer months and restricted nutrition during the winter months. Body condition metrics were collected once per month with biologger data downloaded at the same time.

The study followed a longitudinal design with shifts between reduced and normal diets occurring along four to five-month intervals. Starting in December 2023, elk were given a formulated diet to achieve a roughly 15 percent loss of body mass over four to five months. After four to five months, elk were placed back on their normal diet for an additional four to five months and monitored for body weight gains. Throughout the study, shorter-term treatments were conducted, replicating spikes or rapid reduction of forage access.

All study animals successfully lost weight on the restricted diets over the winter months and then gained weight during the spring and summer months with most animals returning to near-baseline weight by July of 2024. Live animal manipulations for the study are scheduled for completion by September 2024 and data analysis will follow through 2025.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Experimental infection of captive Rocky Mountain bighorn sheep (*Ovis canadensis*) lambs with *Pasteurella multocida*.

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Karen A. Fox, Pauline Nol, Maicie Lingwall, Christopher MacGlover, Kerry Sondgeroth, Lauren von Stade

Respiratory disease has been associated with population declines in Rocky Mountain bighorn sheep (Ovis canadensis) for almost a century[1], and has been well described elsewhere.[1-4] In bighorn sheep, respiratory disease often presents as sporadic outbreaks of bronchopneumonia in all ages[5-8], commonly followed by multiple years of poor neonatal survival.[1,9,10] The cause of respiratory disease in bighorn sheep is multifactorial including bacterial, viral, parasitic, and environmental factors, with complicated interactions and predisposing conditions.[2-4,11]

One component of bighorn sheep respiratory disease is "sinus tumors"; proliferative masses of soft tissue and bone within the paranasal sinuses of the upper respiratory tract in bighorn sheep.[12] These masses have been identified in bighorn sheep herds with a long history of chronic respiratory disease and poor lamb recruitment. The cause of sinus tumors is unknown. We reproduced the disease experimentally by inoculating domestic and bighorn sheep lambs with a cell-free filtrate (to eliminate intact bacteria) made from sinus tumor tissue and exudates.[13] This infection study indicated that sinus tumors are infectious, and the absence of intact bacteria in the inoculum pointed to a viral cause for the disease. However, repeated attempts to identify a viral etiology for sinus tumors have been unsuccessful[14], and trends from naturally occurring cases suggest a possible role for the bacteria *Pasteurella multocida*.

Respiratory pathogen surveillance from respiratory (sinus lining) tissues of bighorn sheep heads (n=273) submitted opportunistically over 13 years suggests an association between P. multocida and sinus tumors. The overall incidence of sinus tumors among the examined bighorn heads is 0.23 (0.18-0.29). Of that overall occurrence, the incidence of sinus tumors in bighorns with *P. multocida* detected in sinus lining is 0.67 (0.54-0.78) as compared to only 0.10 (0.07-0.15) in bighorn heads without *P. multocida* detected in sinus lining tissue (Figure 1). Additionally, preliminary strain typing by MADLI-TOF spectrophotometry demonstrates that *P. multocida* strains associated with sinus tumors are similar to each other, and different from strains unassociated with sinus tumors (Figure 2).

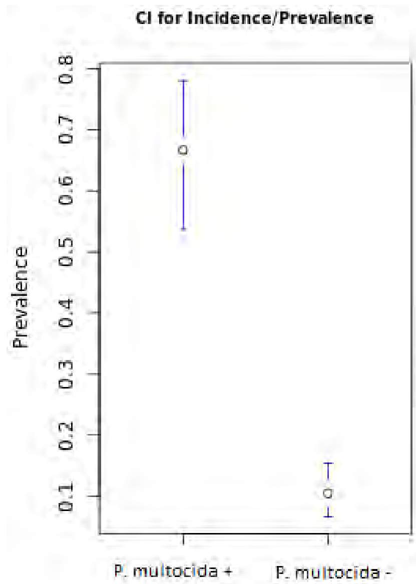

Figure 1. Proportions of bighorn sheep heads (n=273) with sinus tumors detected post-mortem, with (0.67) and without (0.10) concurrent *P. multocida* detected in sinus lining. 95% confidence intervals.

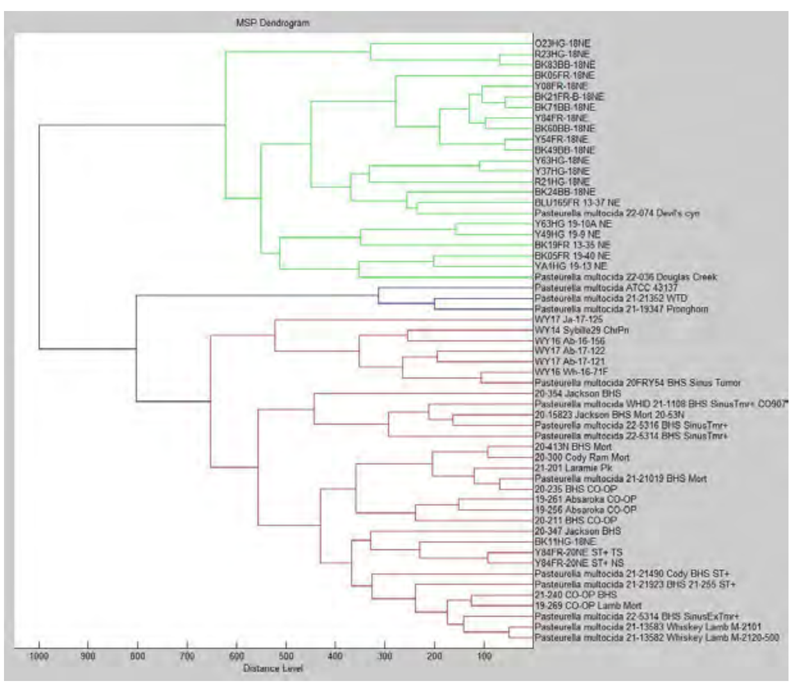

Figure 2. Dendrogram of MALDI-TOF spectrophotometry results, showing strains of *Pasteurella multocida* from apparently healthy animals (green clade) clustering together, and strains of *Pasteurella multocida* from sinus tumors (brown clade) clustering together.

To test our hypothesis that *P. multocida* can induce sinus tumors in bighorn sheep lambs, we will prepare an inoculum from a *P. multocida* strain obtained from a naturally-occurring bighorn sheep sinus tumor (Figure 3). This strain falls within the sinus tumor associated MALDI-TOF clade, and the associated sinus tumor occurred in the absence of other significant respiratory pathogens including *Mannheimia* species, *Bibersteinia trehalosi,* and *Mycoplasma ovipneumoniae*.

Figure 3. Sinus tumor from captive bighorn ram. Note filling of the sinus cavity by white soft tissue, and increased thickness of the surrounding bone trabeculae on the right side of the image. Culture of the soft tissue yielded pure, heavy growth of *Pasteurella multocida*. This culture isolate will be used to produce the inoculum for the proposed study.

Six bighorn lambs were born at the Foothills Wildlife Research Facility in May, 2023. These lambs were pulled from their dams at 24-48 hours of age and were treated with antibiotics to clear respiratory bacteria that the lambs may have been exposed to by their dams. All lambs were initially hand-raised together by caretakers that were dedicated to caring for the six lambs only and did not come in contact with other facility animals or their pens, nor were they to come in contact with any domestic sheep species off site. All facility personnel entering the lamb pen or working with the lambs were not to have entered any other facility pens before entering the lamb pens, and operated under a biosafety protocol that required dedicated clothing, dedicated footwear, and nitrile gloves. From the time of initial separation from the dam through June 2023, nasal swabs were taken bi-weekly to confirm the absence of respiratory bacteria of interest (*Mannheimia* species, *B. trehalosi*, *M. ovipneumoniae*, and *P. multocida*). In July 2023, lambs were separated into groups of three, and housed in two separate enclosures. Three lambs (Treatment) were inoculated intranasally (unilaterally in right nostril) with $1x10^6$ cfu of *P. multocida* in phosphate buffered saline (PBS). Three lambs (Control) were sham inoculated with like volumes of PBS.

We collected nasal and oropharyngeal swabs from all lambs monthly and repeated inoculation for any inoculated animals that fail to produce a *P. multocida* positive swab by culture or PCR. Collection of nasal and oropharyngeal samples were performed with manual restraint or under sedation.

Animals were evaluated daily for evidence of respiratory disease. Any lambs showing evidence of respiratory disease were not re-inoculated with *P. multocida*, even if culture and PCR results are negative for *P. multocida*. Any lambs with evidence of systemic disease unresponsive to treatment would be euthanized and submitted for necropsy examination. CT scans were scheduled to be performed at Colorado State University Veterinary Teaching Hospital at 9 months and 21 months post-inoculation. At any time, clinical signs of a sinus tumor could lead to an unscheduled CT scan. Any CT scans indicating an obstructive or invasive mass would lead to immediate euthanasia and necropsy and examination of the animal. The study is slated for completion in January of 2025, at which time all lambs will be evaluated for evidence of sinus tumor development.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Optimizing chronic wasting disease diagnostics in elk

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Mary E. Wood, Hank Edwards, Jack Grider, Karen Griffin, Jennifer Malmberg, Terry Spraker

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Chronic wasting disease (CWD) is a fatal, infectious disease caused by an abnormal protein called a prion (PrP^d). This disease affects members of the cervid family and is now endemic across much of Colorado. There are currently three United States Department of Agriculture (USDA) approved tests available for CWD diagnosis in cervids through the United States National Animal Health Laboratory Network (NAHLN): immunohistochemistry (IHC), and two enzyme-linked immunosorbent Assays (ELISAs). An additional diagnostic test currently under development and assessment is the real-time quaking induced conversion assay (RT-QuIC).

Challenges with chronic wasting disease diagnostics include lack of DNA or RNA for molecular diagnostic approaches, variation in tissue distribution of the disease, and possible variations in prion distribution among species. Early in the disease course, deposition of CWD prions is sparse and may be limited to certain tissues or even portions of a tissue, resulting in lower diagnostic sensitivity.[1-3] Research evaluating approved diagnostic tests for CWD among different cervid species and tissues is limited, particularly in elk, and there are differences in disease pathogenesis between deer and elk. High-volume field sampling for surveillance and monitoring purposes in free-ranging populations is costly and time-consuming. Identifying diagnostic approaches that utilize a single tissue and diagnostic test will keep surveillance programs cost-effective and efficient.

To better estimate CWD prevalence in Colorado Rocky Mountain elk (Cervus canadensis nelsoni), we evaluated pathogen detectability using different combinations of tissues and diagnostic tests.

Retropharyngeal lymph nodes, tonsils, and obex were opportunistically collected from hunter-harvested elk as well as elk submitted for necropsy in both Colorado and Wyoming in the fall of 2021 and 2022. To discern if an elk was CWD positive, we tested tissues using IDEXX and/or Bio-Rad ELISAs and immunohistochemistry (IHC). We utilized 284 sets of elk tissues for analysis with CWD detected in 41 elk (n=27 females, n=13 males) by at least one tissue and test.

The majority of animals included were female (n=218) since males (n=66) were more likely to be caped or mounted, preventing collection of all samples. Mean age of sampled elk was 5.5 years and mean age of CWD positive elk was 4.22 years, with ages of sampled individuals ranging from 1-21 years. Sampled elk that possessed the leucine allele were predominantly heterozygous (86/89) (Table 1). On six occasions we detected CWD in only one tissue type, with five of those instances occurring in the retropharyngeal lymph node and one occurring in the tonsil. In all cases where CWD was detected in the obex (n=31), it was also detected in another tissue using the same test (RPLN=31, tonsil=21).

We did not find the sex (-0.35 CI: -1.12 – 0.43) or age (-0.18 CI: -0.43 – 0.07 of an individual to influence CWD presence (Figure 7). The presence of the minor leucine allele was found to have a significant influence on CWD susceptibility, with individuals possessing the allele being 5.71 (CI: 2.83-14.72) times less likely to have CWD. We found no effect of test on CWD detection; however, the tissue used significantly affected the detectability of the disease (Figure 7). CWD detection was highest in the lymph node followed by the obex and tonsil, with no significant difference between the obex and the tonsil (Figure 7). Based on model estimates, if an elk population were to have a true prevalence of 5%, using a single tissue sample from the retropharyngeal lymph node, obex, and tonsil would result in prevalence estimates of 4.45%, 3.6%, and 2.57%, respectively (Figure 8).

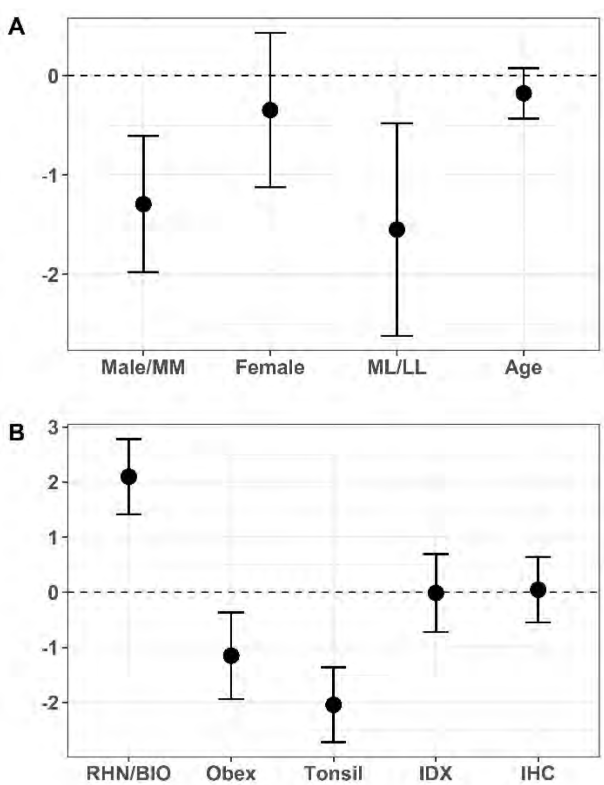

Figure 1: Mean (black dots) and 95% credible interval (back bars) for presence (A) and detection probability (B) of chronic wasting disease in Colorado Rocky Mountain elk (Cervus canadensis nelsoni). Covariates on the far left of plots represent the model intercept and all other covariates are adjustments to the intercept value. In plot A MM and ML/LL represents the absence or presence of the minor leucine allele on codon 132, respectively. Abbreviations in plot B correspond to the retropharyngeal lymph node (RHN), Immunohistochemistry (IHC), IDEXX ELISA (IDX), Bio-Rad ELISA (BIO), and RT-QuIC prepared with tissue from the IDEXX (RT IDX) or Bio-Rad (RT BIO) ELISAs.

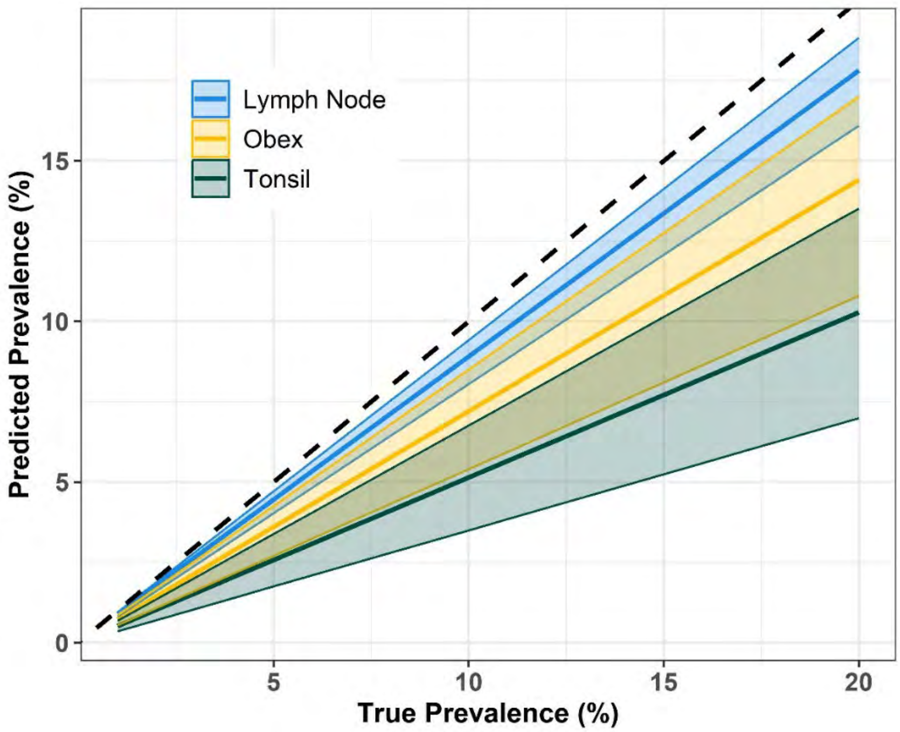

Figure 2: True prevalence (dashed black line) compared to the prevalence that would be observed using retropharyngeal lymph nodes, obex, or tonsil tissue to test for chronic wasting disease in Colorado Rocky Mountain elk (Cervus canadensis nelsoni).

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Experimental infection of Merriam's turkeys with Mycoplasma synoviae

Period Covered: 1 July 2023–30 June 2024

Principal Investigators: Pauline Nol

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Mycoplasma synoviae (MS) is an important pathogen of poultry that can cause respiratory disease, joint infections, egg shell abnormalities, and reduced egg production. Pre-movement testing in Merriam's turkeys in several areas in western Colorado revealed presence of MS in some flocks, and MS has been implicated as a possible factor in population declines in Colorado. Previous work by the wildlife health program has shown equivocal histologic lesions in wild Merriam's turkeys with PCR positive choanal swabs, including mild upper respiratory sinusitis.

At this time, we are unsure whether infected birds pose a risk to naïve wild turkeys or other galliformes. We have identified multiple strains of MS in wild turkey populations in Colorado, but have sampled only a limited number of flocks. Translocation is an important management tool, especially in situations where turkey populations are large and cause conflict with people. Yet, moving birds from infected flocks is currently not recommended to avoid further spread of this bacterium to uninfected bird populations.

We hope to determine whether wild turkeys can develop disease as a result of infection with MS and if they can transmit MS to naïve birds with which they share direct contact. This will serve to better inform CPW managers on proposed translocations of turkeys in Colorado. The results of this study will also inform future research on subsequent challenge models, the possible longer term effects of MS in wild turkeys, and how this pathogen may affect other galliformes in Colorado.

In order to develop an inoculum for MS infection studies, 30 Merriam's wild turkeys were sampled in Meeker, CO from a known infected flock. Seventeen birds were PCR positive for MS. These samples were sent to a collaborator at the University of Georgia for Mycoplasma culture. The laboratory was unsuccessful in growing *Mycoplasma synoviae* from any of the samples sent. There was, however, a novel Mycoplasma species detected, and further investigation is being made in order to characterize this species. In the future, further efforts will be made to obtain a MS inoculum. We are also considering bringing infected birds into captivity in order to conduct a study to examine transmission from naturally infected birds to uninfected birds.

MANAGEMENT AND EDUCATION ACTIVITIES

Veterinary medical activities 2023-2024.

Location of services & primary user	Species	Type of veterinary medical activities
CPW Foothills Wildlife Research Facility (FWRF); Maicie Lingwall & researchers	mule deer, elk, bighorn sheep, pronghorn, bobcat, prairie dogs, others	Preventive, routine, & emergency medical care for all research animals housed at FWRF for use in ongoing CPW research & training.
CPW Frisco Creek Wildlife Facility (FCWF); M. Sirochman	black bear, multiple raptor species	Consultation on preventive, routine, & emergency medical care for rehabilitating wildlife housed at FCWF.
Multiple sites statewide;	elk, moose	Ultrasounded, sampled, & assessed body condition as part of various field research studies. Provided tailored sedation as well as supportive veterinary care for captured animals.
Statewide; wildlife managers, terrestrial biologists	black bear, mountain lion, moose, elk, deer, bighorn sheep, others	Prescribe & track usage of immobilization drugs, recommend & refine dosing instructions, troubleshoot issues related to wildlife immobilization.
Statewide; wildlife managers, terrestrial biologists, researchers	Colorado terrestrial wildlife	Provided capture assistance, equipment, field sampling, medical supplies, and training for various monitoring, translocation, & research projects statewide.
Statewide; wildlife managers, terrestrial biologists, researchers	Colorado terrestrial wildlife	Served on Animal Care and Use Committee as attending veterinarian and wildlife health reviewers.
Wildlife managers, terrestrial biologists, Smithsonian Institute	Swift Fox	Provided veterinary support for capture and translocation of swift foxes to Fort Belknap.
Statewide, wildlife managers, terrestrial biologists	Wild Turkey	Collected samples from turkey capture and translocation efforts for disease surveillance and research.

Training and education provided during 2023-2024.

Location of services & primary user	**Species**	**Type of training**
CPW Foothills Wildlife Research Facility (FWRF); Statewide; wildlife managers, terrestrial biologists	Colorado terrestrial wildlife	Wildlife capture and handling training classes were provided for district wildlife manager trainees, biologists, researchers, and technicians. Capture classes included lectures on drug use regulations and recordkeeping, pharmacology of capture drugs, dosing, safety and types of equipment. *In situ* training was provided at meetings and on-site during various capture operations statewide.
CPW FWRF, veterinary students and interns	Colorado terrestrial wildlife	A 4-week externship was provided for veterinary students in their 4th year of training. Students were exposed to a wide array of experiences related to wildlife medicine and wildlife health and completed directed projects.
Statewide; wildlife managers, terrestrial biologists	Colorado terrestrial wildlife	Chronic wasting disease sampling and data collection trainings were provided to facilitate mandatory CWD sampling in hunter-harvested cervids.
FWRF; Statewide; wildlife managers	Colorado terrestrial wildlife	A wildlife disease and field necropsy training was provided for district wildlife manager trainees. This includes an overview of field necropsy techniques, sample handling and submission, and safety when handling sick wildlife.
Statewide	Colorado terrestrial wildlife	Wildlife disease fact sheets and information support were provided for handling disease-related questions from the public.
Statewide, species conservation and terrestrial biologists, CPW researchers	Gunnison's and white-tailed prairie dogs, black footed ferrets	Training was provided on plague management, bait distribution equipment and duster operation training and maintenance.
Statewide, Park Rangers	Colorado terrestrial wildlife	A euthanasia training course was provided for Park Rangers. This class included lectures on euthanasia methods and provided tools and options for conducting humane wildlife euthanasia.

Disease management during 2023-2024

Location of services & primary user	Species	Type of activities
Statewide; species conservation and terrestrial biologists, CPW researchers	Gunnison's and white-tailed prairie dogs, black footed ferrets	Plague vaccine bait manufacture. Produced ~952,000 baits to vaccinate ~11,400 acres and build freezer stock for future use.
South Park Individual Population Area, conservation and terrestrial biologists	Gunnison's prairie dogs	Vector Control for Plague Management: ~700 acres dusted
Statewide; Terrestrial Resources Program, terrestrial biologists	Native Colorado mammals	Developed guidance for highly pathogenic avian influenza surveillance in coordination with the Colorado Department of Agriculture.
Statewide; regulations manager	Native Colorado cervids	Reviewed cervid import and movement requests in coordination with the Colorado Department of Agriculture.

Laboratory and Diagnostics Provided during 2023-2024

Location of services & primary user	Species	Type of Laboratory Activities
CPW Foothills Wildlife Research Facility (FWRF); Statewide; wildlife managers, terrestrial biologists	Mule deer, white-tailed deer, elk, moose	Chronic wasting disease surveillance and monitoring. Facilitated testing of over 3,700 samples for CWD
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Black-tailed prairie dogs, white-tailed prairie dogs, Gunnison's prairie dogs, multiple flea species	Plague testing of tissue samples and flea pools 64 carcasses 695 flea pools
FWRF; Statewide; managers, terrestrial biologists, researchers	Cottontails, jack rabbits, snowshoe hare	RHDV2 testing of 22 tissue samples
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Avian spp.	Trichomonas testing of 6 tissue samples.
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Bighorn sheep	Testing of tissues from 55 cases for respiratory disease pathogens.
Statewide; wildlife managers, terrestrial biologists, researchers	Felids, rodents	Rustrela virus testing of 4 samples
FWRF; Statewide; district & area wildlife managers, terrestrial biologists, researchers	Wide variety of terrestrial wildlife species.	Serum and tissue banking. 448 samples added to existing serum and tissue banks.
CPW researchers and CPW animal care and use committee	Wide variety of terrestrial wildlife species.	Conducted necropsies for statewide wildlife disease surveillance or to determine cause- specific mortality for various ongoing research projects.

Summary of Necropsy Submissions 2019-2023

Species	2019	2020	2021	2022	2023
Avian	96	59	72	190	120
Badger	0	0	0	0	0
Bat	84	70	51	17	5
Bear	11	16	9	4	5
Beaver	1	1	0	0	1
Bighorn sheep	36	23	58	48	55
Black footed ferret	0	0	0	0	0
Bobcat	0	0	1	0	6
Coyote	5	0	2	0	0
Domestic	3	8	1	0	0
Elk	33	42	40	24	36
Fish	0	0	0	0	0
Fox	1	6	3	2	5
Lynx	0	0	0	0	0
Marmot	0	1	1	0	0
Marten	0	0	0	0	0
Mink	0	1	0	0	0
Moose	6	6	2	5	8
Mtn goat	3	1	0	0	1
Mtn lion	23	14	10	13	19
Mule Deer	56	46	59	34	23
Muskrat	0	0	0	0	1
Porcupine	0	0	0	0	0
Prairie dog	4	4	25	0	7
Pronghorn	1	3	2	3	2
Rabbit/Hare	3	62	35	31	13
Raccoon	9	10	2	2	5
Ringtail	0	0	0	0	0
River Otter	0	0	0	0	2
Skunk	1	3	0	1	6
Sm Rodents	4	12	10	6	3
White-tailed deer	2	3	3	0	8
Totals	382	397	386	380	331

WILDLIFE HEALTH PROGRAM PUBLISHED AND IN PRESS MANUSCRIPTS DURING 2023-2024

Published FY 2023-24

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