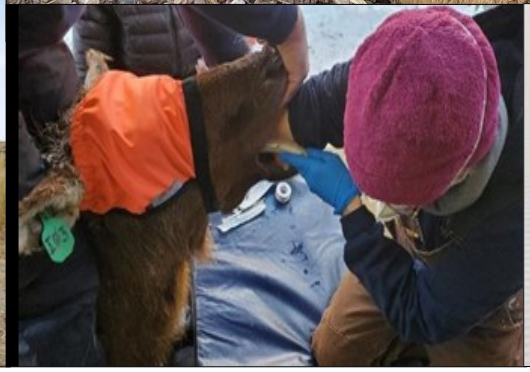
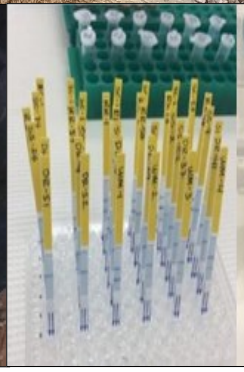
COLORADO PARKS & WILDLIFE

Wildlife Research Reports

Wildlife Health

July 2020—June 2021





WILDLIFE RESEARCH REPORTS

JULY 2020-JUNE 2021

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 conducted by the wildlife health program of Colorado Parks and Wildlife (CPW) from July 2020 through June 2021. 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 support 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 and plague in prairie dogs and black-footed ferrets. Chronic wasting disease work focused heavily on identifying management opportunities to limit the disease in free ranging cervid populations and answering lingering questions on non-cervid species susceptibility to CWD. Plague research focused on working toward better understanding of the prediction and control of plague epidemics in prairie and shrub-steppe systems. 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 evaluation of drug combinations to facilitate safe capture of wildlife.

Support services during 2020−2021 continued to emphasize detection of important wildlife health problems via examination of field case submissions, along with site visits and field investigations as warranted. New challenges in 2020-2021 included the emergence of Rabbit Hemorrhagic Disease Virus type 2 (RHDV-2), a foreign animal disease affecting lagomorphs, and addressing risks associated with SARS-CoV-2, the virus responsible for Covid-19, in wildlife populations. The wildlife health program also provided training, field, and laboratory support for many other CPW management and research programs.

In addition to these more general support services, 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.

Our work was supported by numerous collaborators. 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 zoonotic and otherwise important diseases. 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, World Wildlife Fund, Advantage Bio Consultants, Species Conservation Trust Fund, Wildlife Pharmaceuticals, Texas A&M Veterinary Diagnostic Laboratory, Wyoming State Veterinary Laboratory, Alberta Fish and Wildlife, Nebraska Game and Parks, Utah Division of Wildlife Resources, and the Wyoming Game and Fish Department.

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WILDLIFE RESEARCH PROJECT SUMMARY

Prion degradation & pass-through in mountain lions

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Michael W. Miller, Lisa L. Wolfe, Brent Race, & Chase Baune

We conducted a small, opportunistic study to quantify changes in the seeding activity of prions passed through the digestive tract of mountain lions (*Puma concolor*). In conjunction with a long-term study of natural chronic wasting disease (CWD) susceptibility in captive mountain lions, we collected samples to examine prion concentrations in feces passed after consuming a known dose (brain homogenate) of CWD-positive material. Prior to feeding the brain homogenate, the two available mountain lions received a carcass that was CWD-not detected (ND) to reduce the amount of background infectious material in their gut. At the next scheduled feeding one animal received 0.5 kg deer meat that was CWD-Not Detected and the other received CWD-Not Detected deer meat laced with ~50 g of CWD-positive brain homogenate verified by IHC and ELISA (OD value = 3.405). Once treated, the mountain lions were separated by a fence barrier to facilitate collecting each individual's feces over a 7-day period. Feces from both individuals were collected (as available) for 1 day pre- and 7 days post-inoculum ingestion and frozen at −80°C until analysis. Six weeks later, we switched treatments and repeated the exposure and sampling as described. Laboratory analyses are underway.

Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Assessing harvest management influences on chronic wasting disease trends in the West

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Mary M. Conner, Mary E. Wood, Anne Hubbs, Justin Binfet, A. Andrew Holland, Luke R. Meduna, Annette Roug, Jonathan P. Runge, Todd D. Nordeen, Margo J. Pybus, Michael W. Miller

There is an urgent need to identify practical management strategies for chronic wasting disease (CWD) that can be implemented in a long-term, sustainable manner to minimize impacts on cervid resources nationwide. Chronic wasting disease threatens the health and viability of native cervid populations in North America and elsewhere, but capacity to effectively contain and suppress epidemics remains lacking.[1-3] Prior control attempts have met with limited success, and most were abandoned within a few years due to high personnel/agency costs, inability to demonstrate efficacy, and lack of public support. Prevalence and distribution continue to rise in most affected jurisdictions. Local effects on deer and elk herd viability have been reported,[4,5] and accumulating evidence suggests more widespread impacts seem likely if effective control strategies cannot be identified.

We coordinated a multi-jurisdictional effort to assemble and synthesize available, long-term data on herd management and CWD trends from cooperating Western jurisdictions to develop guidance on harvest-based control strategies. Five agencies participated in this project: Alberta Environment and Parks, Colorado Parks and Wildlife, Nebraska Game and Parks, Utah Division of Wildlife Resources, and Wyoming Game and Fish Department.

We performed logistic regression analyses and model selection from retrospective data on harvest management practices and chronic wasting disease surveillance. A total of 36 management units across the five jurisdictions met the data requirements for inclusion in the project. All competitive models included the number of male deer harvested or number of hunters 1−2 yr prior as an explanatory variable, with increasing harvest leading to lower prevalence among males harvested in the following year. Competitive models also included harvest timing. Although less definitive than the number harvested, median harvest dates falling closer to breeding seasons were associated with lower prevalence in the following year. Our

findings suggest harvest can be an effective tool for attenuating CWD prevalence in adult male mule deer across western ranges, especially early in the course of an epidemic.

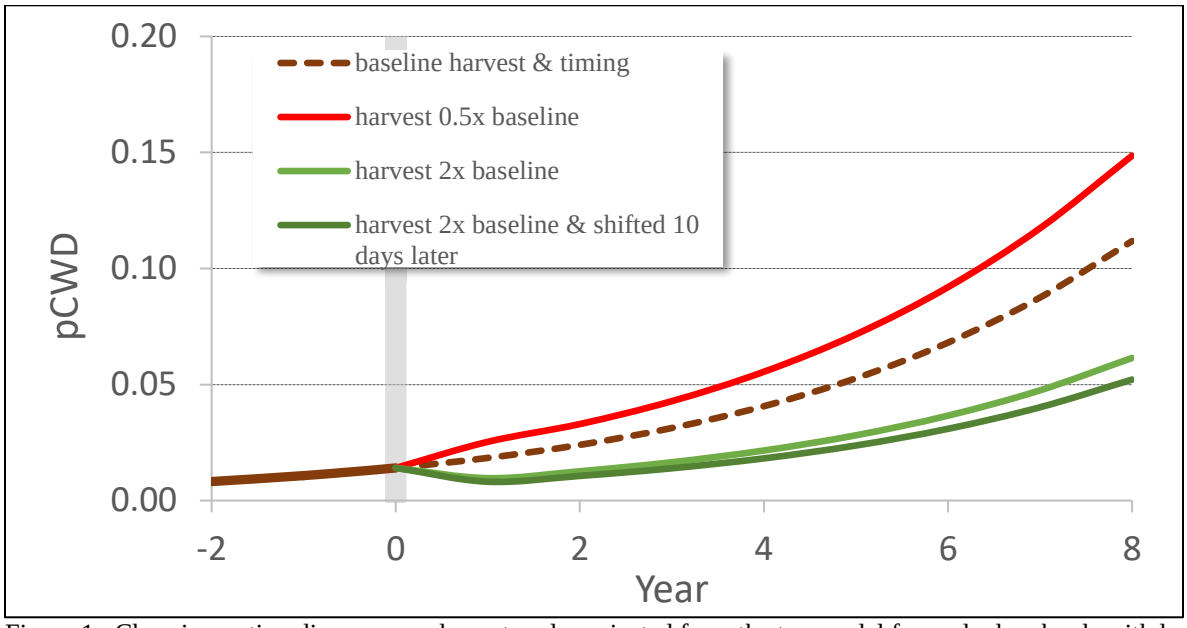

Figure 1. Chronic wasting disease prevalence trends projected from the top model for mule deer herds with low starting prevalence. Trend lines illustrate the relative influence of changes in annual harvest amount or timing beginning at Year 0 (vertical gray bar) as compared to maintaining baseline harvest (dotted line).

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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 2020–30 June 2021

Principal Investigators: Dan Tripp

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 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. Six release sites have been identified since 2013 with ~400 ferrets released and additional sites could be identified in the near future. Unfortunately, only two of these six reintroduction sites are still active with the remainder 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 needed to sustain the reintroduced population of 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] In 2017-19, about 1700 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.[5]

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 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[6]. 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 light of these findings, further investigation of free-ranging prairie dogs' antibody responses to vaccine dosage and seasonal timing of vaccination is needed. Current baiting strategy is to apply vaccine in the autumn when bait uptake is superior to baiting in the spring.[7] Advantages of autumn baiting include the presence of older, more adventurous juveniles, who are more likely to encounter and compete with adults for baits, fewer juveniles competing for baits as their survival declines during first year of life and a reduction in competition for prairie dogs' interest between baits and abundant green grass.[8, 9]

Despite the advantages of autumn baiting, spring baiting may be beneficial for limiting plague transmission when the prairie dog population is inundated with a new cohort of unvaccinated juveniles. Additionally, it has been demonstrated that survival of young vaccinated prairie dogs was superior to vaccinated adults when challenged with plague in the laboratory.[10] This advantage may be amplified if prairie dogs consume vaccine baits within ~1 month of emergence (i.e. 4 months earlier than the current strategy for black-tailed prairie dogs). Targeting young black-tailed prairie dogs for vaccination in the spring may further increase population-wide plague suppression by shortening the length of time that the juvenile cohort is unprotected. This vaccination strategy may amplify vaccine efficacy in black-tailed prairie dogs by decreasing plague transmission among juveniles while also providing a "booster" dose of vaccine to previously vaccinated adults.

Methods

Study areas (4) included plots/colonies at Soapstone Prairie Natural Area, owned by the City of Fort Collins. Final selection of study colonies and plots followed field observations conducted in May-June 2020 to determine habitat quality and prairie dog population health. We included two paired plots (spring and autumn) at sites SNA-01 and SNA-12 (Fig. 1 and Fig. 2).

Despite the global Covid-19 pandemic and accompanying travel restrictions, we were able to conduct fieldwork as planned. Many thanks are due the dedicated CPW crews that continued to perform professionally despite the many challenges and safety concerns that 2020 presented.

In 2020, we captured and sampled free-ranging black-tailed prairie dogs on plots at the Soapstone Prairie and Meadow Springs Ranch complex, where we administered 8x dose vaccine baits in either spring or autumn. This design provided serology data to compare antibody responses from prairie dogs captured on plots receiving 8x vaccine baits in spring or autumn. We will also compare antibody responses to 8x dose baits with similar data collected during a dose-response field study conducted in 2017-18.

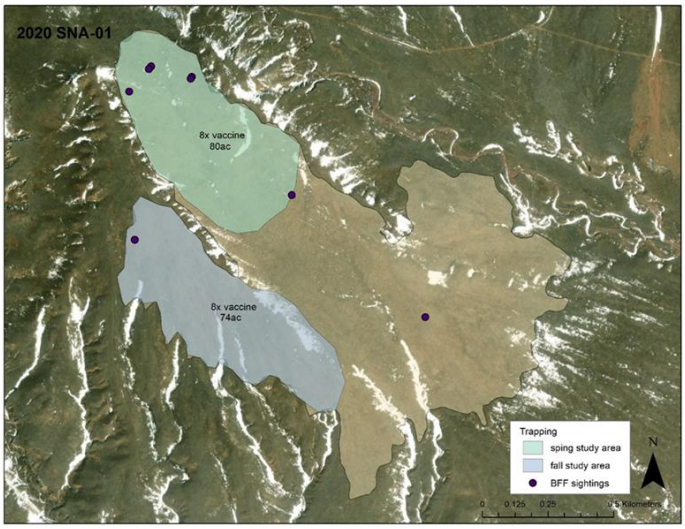

Figure 1. Treatment area at the Soapstone Natural Area Colony SNA-01. Spring (green) and autumn (blue) 8x vaccine treatment areas are shown.

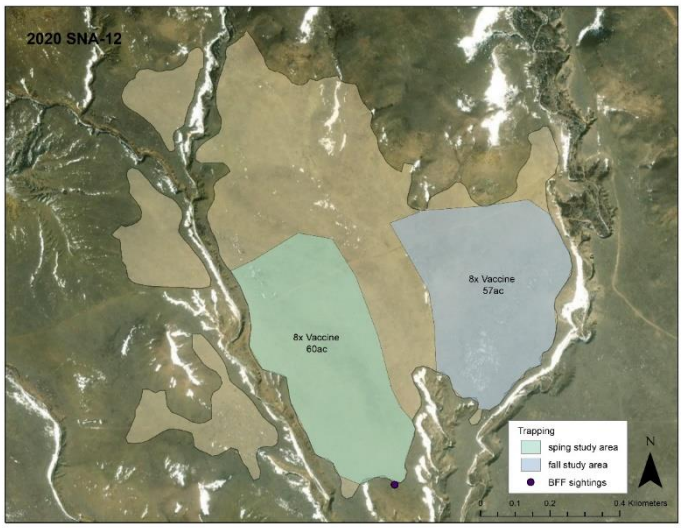

Figure 2. Treatment area at the Soapstone Natural Area Colony SNA-12. Spring (green) and autumn (blue) 8x vaccine treatment areas are shown.

Preliminary Results

We conducted prairie dog occupancy and burrow activity surveys on 38 colonies/plots at the Soapstone Prairie and Meadow Springs Ranch complex.[4] We distributed ~257,000 doses of vaccine in ~90,700 baits on ~1,814 acres at the Soapstone Prairie and Meadow Springs Ranch complex. We also applied experimental vaccine baits (8x dose) on 271 acres spanning four research plots (Figure 1, 2) and captured and sampled 182 prairie dogs on these plots in 2020.

In the spring, 48% of the captured prairie dogs were seropositive for the V antigen (stimulated by the vaccine) on the SNA-01 plot while 28% were seropositive on the SNA-12 plot (Figure 3). In the autumn, 29% of the captured prairie dogs were seropositive for the V antigen on the SNA-01 plot while 42% were seropositive on the SNA-12 plot (Figure 3).

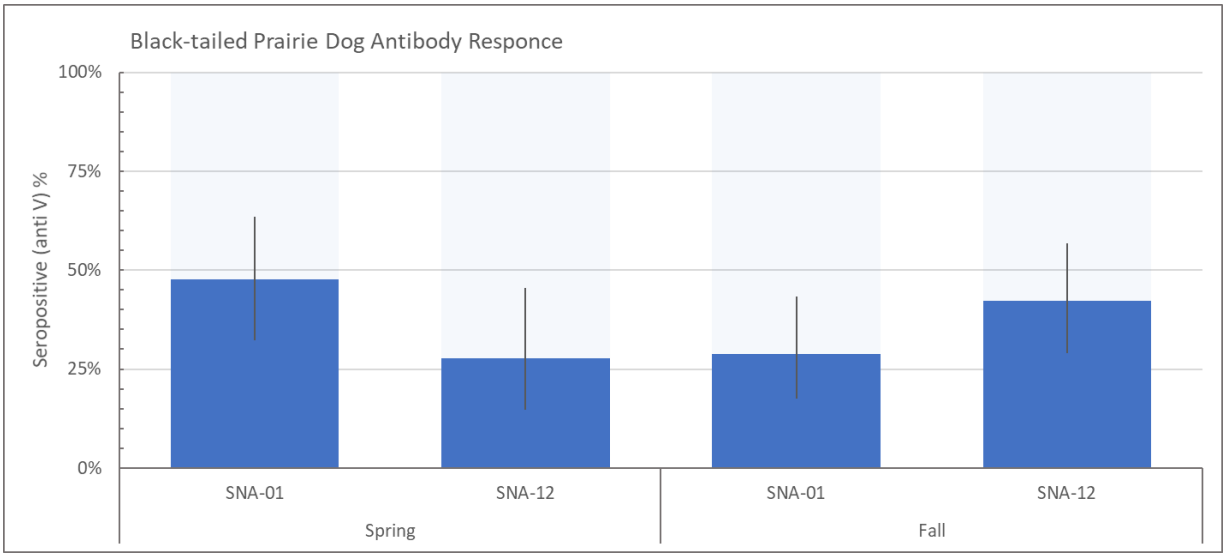

Figure 3. Proportion of vaccinated prairie dogs with detected serum antibodies to *Yersinia pestis* V antigen ("seropositive") 28–38 days after receiving vaccine (RCN-F1-V307) orally at the dose of ~4.8×10^8 $TCID_{50}$ ("8x").

We conducted prairie dog occupancy and burrow activity surveys in the spring and autumn on 38 colonies/plots receiving combinations of dust, vaccine and no treatment at the Soapstone Prairie and Meadow Springs Ranch complex in 2020 (Figure 4). We will use these data to monitor responses to plague management though time.

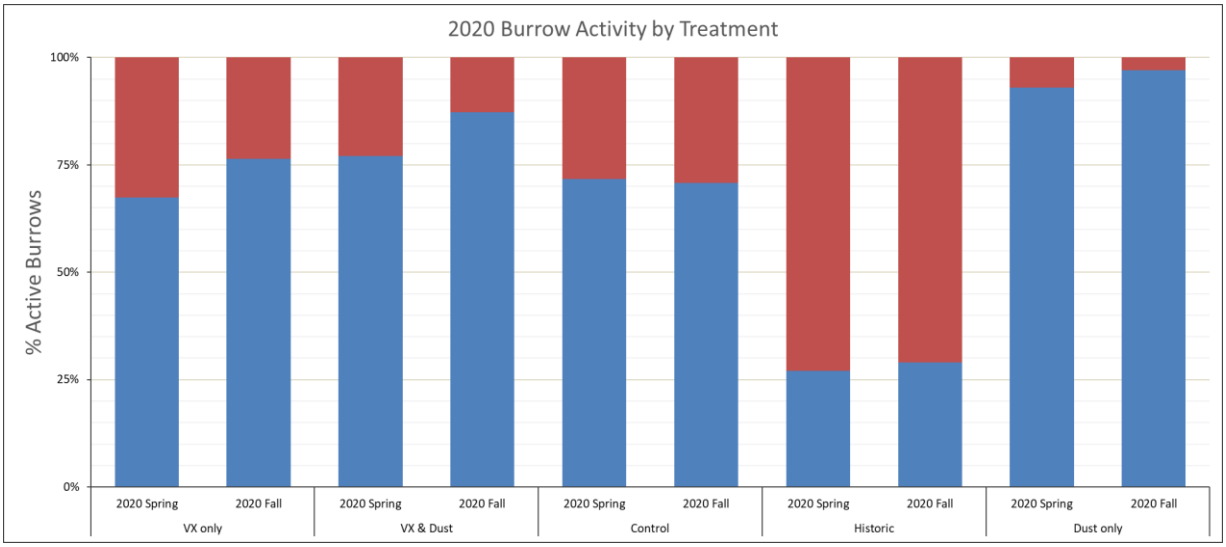

Figure 4. Proportion prairie dog burrows scored as active (blue) and inactive (red) during surveys conducted in the spring and autumn 2020 on colonies receiving plague management at the Soapstone Prairie and Meadow Springs Ranch complex.

We also assisted the City of Fort Collins Natural Areas program with spotlight surveys for black-footed ferrets in October 2020. Our CPW crew operated a spotlighting vehicle for 5 nights (~50 hours). This effort significantly expanded the area surveyed.

Future Efforts

We will continue to analyze data collected in 2020 and prepare for continued field research in 2021. In 2021, we will continue vaccine bait manufacture, monitoring efforts and research to optimize the vaccine dose/bait and baits/acre to maximize seroconversion and cost efficiency of vaccination. Optimizing the vaccine dose and baiting rate may maximize bait uptake and seroconversion while accounting for the likelihood that some individuals (adult females) may consume multiple baits. This optimization may also increase bait uptake in juvenile prairie dogs, which is needed to provide greater population-level protection from plague.

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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 2020–30 June 2021

Principal Investigators: Dan Tripp

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.[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 ~1500-2000 acres of black-tailed prairie dog colonies annually. An additional ~1500-2000 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. 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 the additional untreated ~1500 - 2000 acres while also continuing to evaluate oral plague vaccine 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 ~1500 to

2000 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] 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 (Fig 1). In addition to sustaining an annual plague management effort, we have continued to evaluate and adaptively use oral plague vaccine 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 2016-20.

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

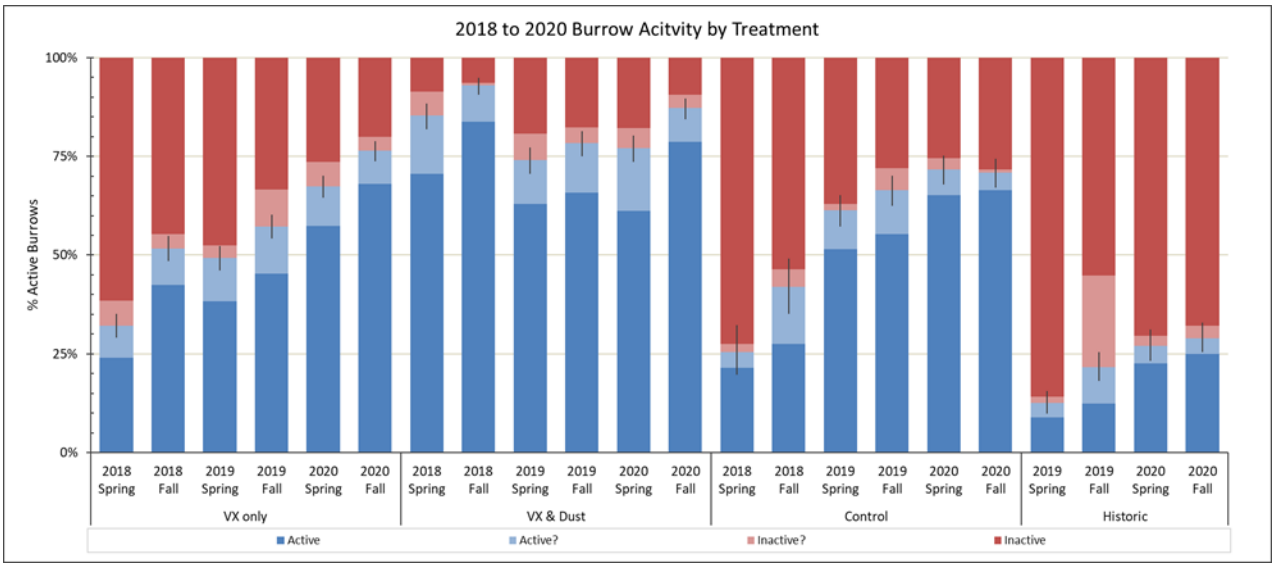

Figure 1. Proportion prairie dog burrows scored as active (blue), likely active (light blue), inactive (red) and likely inactive (light red) during surveys conducted in the spring and autumn 2018-2020 on colonies receiving plague management (vaccine, dust) or no treatment (control, historic) at the Soapstone Prairie and Meadow Springs Ranch complex.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Assessment of gastrointestinal transit time and fecal staining after consumption of placebo baits in captive black tailed prairie dogs

Period Covered: 01 July 2020 – 30 June 2021

Principal Investigators: Daniel W. Tripp, Danielle A. Sack, Alexis C. Emslie, Maicie L. Sykes

Plague, caused by the bacterium *Yersinia pestis*, has contributed significantly to the overall decline of Colorado's three prairie dog species and has hampered efforts to prevent extinction of the black-footed ferret.[1,2] Because understanding and controlling plague is considered essential for successfully conserving these and other wildlife species, Colorado Parks and Wildlife's (CPW) guiding strategies[2,3] have placed emphasis on plague management and research.

A recombinant oral vaccine against plague (RCN-F1/V307)[4,5]; was developed by the US Geological Survey's National Wildlife Health Center (NWHC) and appears to be safe and effective in at least two species of prairie dogs under field conditions[6,7] (CPW unpublished data). In Colorado field studies, prairie dogs residing in vaccinated plots showed higher survival and persistence in the face of naturally occurring plague epizootics than prairie dogs in unvaccinated control plots that invariably collapsed once struck by plague.[7] As a result, oral vaccination of prairie dogs has emerged as a potential viable alternative to burrow dusting as a plague control tool.[7-9]

Prairie dogs that consume vaccine baits produce feces that is stained blue by food dye that is incorporated into baits during manufacture (FD&C Blue 1 food dye; Fig. 1).[10] In the field, this stained feces is conspicuous and its presence on baited colonies after vaccine bait distribution, indicates recent prairie dog occupancy, activity and consumption of vaccine baits by the target species.

However, the gastrointestinal transit time, amount and variation in color of fecal staining is currently unknown. Thus, our ability to understand how the presence and amount of stained

feces on field sites relates to the number of prairie dogs that consumed bait or the number of baits consumed by each animal is limited.

In this pilot study, we opportunistically evaluated the gastrointestinal transit time, amount and variation in color of fecal staining in eight individual captive black-tailed prairie dogs that voluntarily consumed placebo baits. We found that fecal pellets were visibly stained 24 hours after bait consumption and dark, saturated staining was visible for up to 72 hours (Table 1, Fig. 1). Some study animals reduced their food intake because of the increased animal care and feces collection activity. Therefore, their gastrointestinal transit time may have been delayed. Thus, the timing of fecal pellet staining after bait consumption in captive prairie dogs may not be a reliable proxy for free ranging prairie dogs. We concluded that free ranging prairie dogs would likely begin to produce blue stained fecal pellets 24 hours after vaccine bait consumption (Table 1). However, we are not likely to accurately differentiate between fecal pellets produced between 24 and 96 hours post consumption or between prairie dogs that consume a single or multiple baits (Fig. 1).

Table 1. Fecal pellet count and percentage of fecal pellets with blue staining in captive prairie dogs after consuming one or two placebo baits.

	1 bait consumed		2 baits consumed	
Hours post bait consumption	Pellet Count	Fecal pellets with blue staining (%)	Pellet Count	Fecal pellets with blue staining (%)
0	0	0%	0	0%
24	79	45%	131	76%
48	81	83%	185	78%
72	223	88%	133	21%
96	94	82%	105	13%

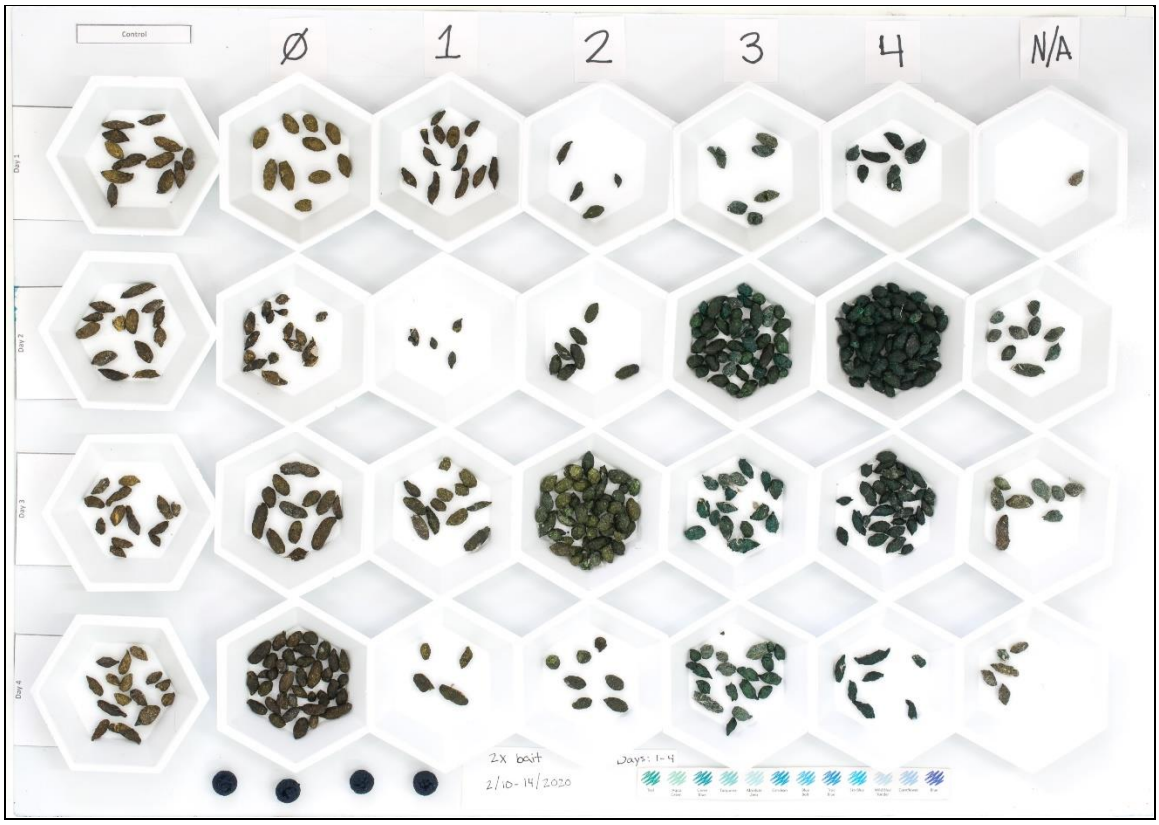

Figure 1. Fecal pellets collected from captive prairie dogs and scored for blue staining (0-4, lightest to darkest) at 24, 48, 72, and 96 hour after consuming two placebo baits.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Assessing safety & antibody responses of black-tailed prairie dogs to oral *Yersinia pestis* Vaccine construct RCN-F1-V307-RovM

Period Covered: 01 July 2020 – 30 June 2021

Principal Investigators: Michael W. Miller and Pauline Nol

Plague epidemics in prairie and shrub steppe ecosystems cause dramatic reductions in prairie dog and black-footed ferret populations.[1] Plague has contributed significantly to the overall declines of Colorado's three prairie dog species and has hampered efforts to prevent extinction of the black-footed ferret.[1] Consequently, understanding and controlling plague has emerged as a critical need for conserving reintroduced black-footed ferrets and imperiled prairie dog populations.[1]

Oral vaccination has become a mainstay of species conservation efforts involving plague suppression in Colorado.[1] The "Yersinia Pestis Vaccine" (Colorado Serum Company, Denver, CO) now in use is a recombinant raccoon poxvirus (RCN) genetically altered to express two *Yersinia pestis* antigens, F1 and a truncated V protein (denoted as V307).[2] The conditionally licensed construct is denoted herein as RCN-F1-V307. Laboratory and field studies showed that consuming RCN-F1-V307-laden baits before exposure to plague improved survival of prairie dogs (*Cynomys* spp.), but protection is not always complete.[2,3] Improvements to this vaccine may improve its effectiveness in helping meet management goals for species of concern in Colorado.

Expression of F1 is considerably lower than of V307 in the RCN-F1-V307 construct, resulting in lower anti-F1 antibody responses among vaccinated prairie dogs.[2,4] Although the conditionally licensed construct is protective, high levels of anti-F1 antibodies observed in prairie dogs surviving natural plague exposure suggest that better stimulation of F1 responses could enhance overall efficacy in field applications. Since the RCN-F1-V307 construct was created, new techniques to improve expression of multiple gene insertions have been developed. Better F1 and V307 target gene expression should enhance antibody responses in prairie dogs.

In addition to suboptimal F1 gene expression, the RCN-F1-V307 construct does nothing to directly reduce *Y. pestis* loads in fleas because neither the F1 nor the V protein are expressed within fleas. Different *Y. pestis* proteins are expressed in fleas at their lower body temperatures. Proteins that facilitate the bacteria's ability to colonize flea gut and to form biofilm (e.g., Ymt

and Hms) appear especially key to successful flea infection. Immunizing host animals against these proteins might stimulate host serum antibodies to interfere with bacterial colonization in fleas that feed on the vaccinated hosts. This strategy has been employed successfully to vaccinate small rodents against Lyme disease with antigens that target proteins responsible for growth of the pathogen in tick vectors.[5–7]

We conducted a study in captive prairie dogs to demonstrate safety and assess immunogenicity of an unlicensed recombinant RCN-vectored vaccine expressing the *Y. pestis F1* (capsular antigen) gene, a truncated *lcrV* gene (V307), and the *RovM* gene (expressed during flea infection) – denoted herein as RCN-F1-V307-RovM – after oral delivery via edible baits. Vaccine baits carried approximately 5×10^7 $TCID_{50}$. We detected no adverse reactions to oral vaccination with RCN-F1-V307-RovM. Resulting serological data indicated that 11 of 12 animals that consumed an entire bait developed antibodies to V307. In contrast, only two of 12 vaccinated animals developed detectible antibodies to F1. Six vaccinates developed antibodies to RovM. This suggests an improved antibody response to V307 as compared to previous vaccine studies, as well as successful elicitation of immune response to RovM by this new construct. However, there was no increase in detected antibody against F1.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Assessing post-production ethylenediaminetetraacetic acid (EDTA) treatment of Yersinia Pestis Vaccine solution to improve antibody responses in black-tailed prairie dogs

Period Covered: 01 July 2020 – 30 June 2021

Principal Investigators: Daniel W. Tripp, Pauline Nol, Karen A. Griffin, and Keith N. Haffer

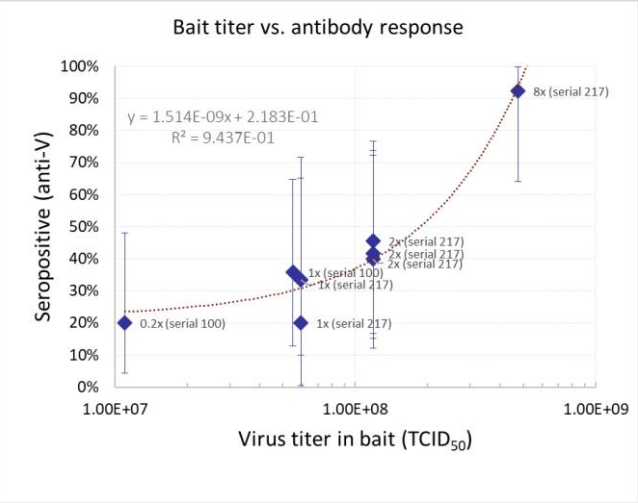
Plague epidemics in prairie and shrub steppe ecosystems cause dramatic reductions in prairie dog and black-footed ferret populations.[1] Plague has contributed significantly to the overall declines of Colorado's three prairie dog species and has hampered efforts to prevent extinction of the black-footed ferret.[1] Consequently, controlling plague has emerged as a critical need for conserving reintroduced black-footed ferrets and imperiled prairie dog populations.[1]

Oral vaccination has become an important tool for dampening plague effects in select prairie dog populations in Colorado.[1] The conditionally licensed *Yersinia Pestis* Vaccine (YPV; Colorado Serum Company, Denver, CO) in field use since 2017 is a recombinant raccoon poxvirus (RCN) genetically altered to express two *Yersinia pestis* antigens, F1 and a truncated V protein, denoted as RCN-F1-V307.[2,3] Consuming RCN-F1-V307-laden baits before exposure to plague improves survival of prairie dogs (*Cynomys* spp.) in laboratory and field studies, but complete protection is unlikely.[2–4]

Available data suggest the effectiveness of extant plague vaccination efforts could be improved by modifying vaccination strategies. In particular, prior work has shown that the proportion of prairie dogs with detectable serum antibodies to *Y. pestis* V antigen (anti-V) depends on the dose of vaccine administered (Figure). The YPV label dose – 5×10^7 plaque forming units ("1×")[3] based on the median tissue culture infectious dose ($TCID_{50}$) measured via an industry- and regulatory-standard 6-day end-point count – stimulates detectable antibody responses in only about one in three vaccinated prairie dogs on average (Figure). Progressively higher vaccine doses increase the proportion of animals responding (Figure), but that improvement comes at a progressively higher cost under current vaccine bait manufacturing practices.

Recent *in vitro* work with YPV (Haffer unpublished) 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 is believed to aid in breaking up large aggregates of live RCN-F1-V307, thereby making more individual virus particles biologically available. If this improved bioavailability transfers to *in vivo* conditions then the proportion of prairie dogs with detectable antibody responses would be expected to increase (perhaps ~2-fold or more) with negligible increase in operational costs.



We evaluated whether EDTA treatment of YPV as a modification to in-house vaccine bait manufacturing protocols can elicit measurable improvement in antibody responses among orally vaccinated prairie dogs compared to established standards (Figure). Of 12 prairie dogs that consumed an entire vaccine bait, 11 developed detectable antibodies against V antigen. Two of three animals that consumed partial baits also developed anti-V antibodies. All animals were sampled between 22 and 56 days post-vaccination. The animal that consumed a full bait but was negative for anti-V antibodies was sampled at 44 days and another negative animal that ate ¾ of a bait was sampled on day 28. None of the prairie dogs developed any detectable antibodies to F1 antigen. We detected no adverse reactions to oral vaccination with RCN-F1-V307 treated with EDTA. Further studies will be initiated to evaluate this vaccine preparation in the field

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Analysis of genomic data for bighorn sheep respiratory disease diagnostics

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Karen A. Fox, Kevin Blecha

Wildlife disease diagnostics are evolving to encompass new methods of pathogen detection and identification. As DNA amplification and sequencing have become faster and cheaper processes, researchers can now specifically and nonspecifically sequence large amounts of DNA from tissue samples. Through these methods, often called next-generation sequencing (NGS), large data sets are generated. The analyses of these data sets are currently a focus of emerging technology and research. NGS can be used to identify new pathogens, and more thoroughly describe and categorize existing pathogens. These abilities are powerful tools to complement traditional diagnostics.

Bighorn sheep respiratory disease is a polymicrobial disease that may be better understood through NGS methods. Contact between domestic and bighorn sheep is a consistently demonstrated risk to bighorn health[1,2] although natural transmission of pathogens is difficult to demonstrate. Strain typing of *Mycoplasma ovipneumoniae* has been used to demonstrate novel pathogen introduction into bighorn sheep herds,[3] although demonstrating a source from domestic sheep is difficult in part due to the mixture of strains found in any domestic flock. In 2018, contact was documented between a free-ranging bighorn sheep and a domestic sheep that was later culled and demonstrated to have chronic respiratory disease (Kevin Blecha, Karen Fox unpublished data). The bighorn sheep died from fatal acute bronchopneumonia after contact. We will use multiplex PCR (Figure 1) and NGS methods to examine tissues from both animals. This assay targets multiple genes that can classify the bacteria beyond the scope of current diagnostics (Figure 2). This assay can now be applied to samples from across the state to better categorize the pathogens present in bighorn sheep herds, and understand pathogen transmission and epidemiology.

One component of bighorn sheep respiratory disease is sinus tumors, for which a causative agent has not yet been identified.[4] Work is ongoing to use NGS methods to identify

possible viral pathogens in tissues from affected animals. Early work has targeted an envelope protein found in some bighorns with sinus tumors (Fox, 2012).

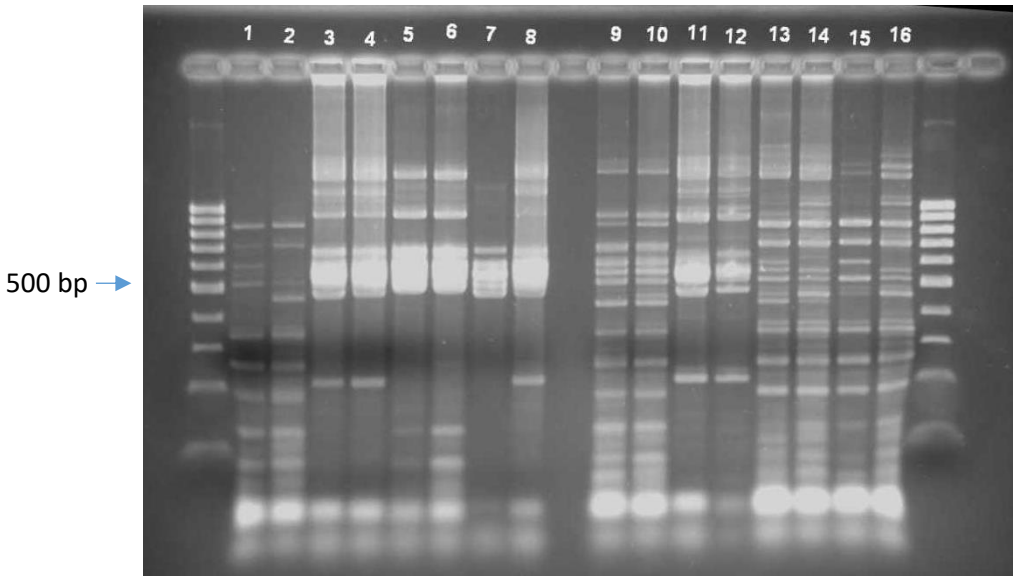

Figure 1. PCR gel showing amplification of (up to) 31 PCR products for bighorn sheep bacterial pathogen multiplex. Note very bright bands near 500 base pair (bp) marker. This indicates samples for which the PCR reaction has amplified abundant products of the desired length.

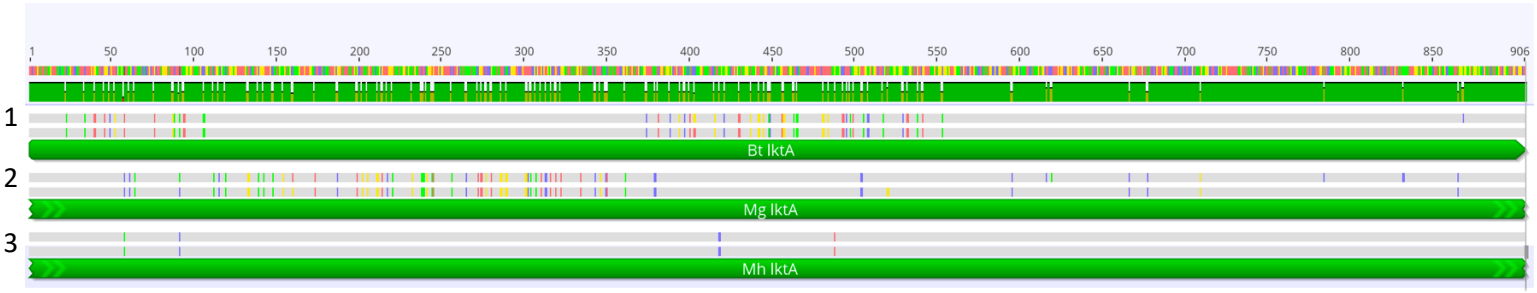

Figure 2. One example of an alignment resulting from data generated by the bighorn sheep PCR multiplex with NGS sequencing. In this case only the Pasteurellaceae leukotoxin gene is displayed. Leukotoxin (lkt) is only one of 27 genes that were sequenced across four pathogens. In this figure, results from each of the three samples are shown. The vertical, colored lines represent areas where the leukotoxin genes are different between the three species. Matching colors represent the same base at that location. The leukotoxin gene from Sample 1 is identified as *Bibersteinia trehalosi* lkt based on similarity to the reference sequence - shown directly below the sequence data from Sample 1. Likewise, the lkt gene from Sample 2 was identified as *Mannheimia glucosida* lkt, and the lkt gene from Sample 3 was identified as *Mannheimia hemolytica* lkt. This is one example of how this assay improves on existing diagnostics which incorrectly identified Sample 2 as containing *Mannheimia hemolytica*. This sample was identified as containing *Mannheimia glucosida* across all genes identified.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Efficacy of tolazoline and vatinoxan in reducing adverse effects of butorphanol-azaperone-medetomidine in Rocky Mountain elk.

Period Covered: 01 July 2020 – 30 June 2021

Pauline Nol, Annette Roug, and Khursheed Mama

In North America, hoofstock such as Rocky mountain elk (*Cervus elaphus nelsoni*), deer (*Odocoileus hemionus* and *Odocoileus virginianus*), bighorn sheep (*Ovis canadensis*), or moose (*Alces alces*) are commonly immobilized with a combination of butorphanol, azaperone, and medetomidine (BAM), or the newer combination nalbuphine, azaperone, and medetomidine (40 mg/ml nalbuphine, 10/ml medetomidine, and 10 mg/ml azaperone, NalMedA).[1-5] While requiring relatively small volumes, providing reasonably short induction times, and smooth recoveries, these combinations frequently causes severe bradycardia, hypertension, low respiratory rates, and hypoxemia.[2-5] There is a need to evaluate easily implementable solutions to improve the cardiovascular and respiratory adverse effects of these combination when used to immobilize wildlife.

The main immobilization drug in these combinations is medetomidine, an alpha-2 adrenergic agonist. After administration, alpha-2 agonists initially cause vasoconstriction and reflex bradycardia (decreased heart rate) followed by a decrease in systemic vascular resistance through vasodilation with continuous low heart rate.[6] After the initial vasoconstriction and hypertension, animals become bradycardic, and further, the alpha-2 adrenergic agonists can cause reduction in ventilation leading to hypoxemia.[6] For example, a study in white tailed deer immobilized with BAM showed that the mean (+/- standard deviation) arterial partial pressure oxygen level was 41.9 (8.9) mmHg, and the level did not improve throughout the immobilization without oxygen supplementation.[3]

Alpha-2 adrenergic agonists such as medetomidine are completely reversible with atipamezole, which is a competitive antagonist with strong affinity for central and peripheral alpha-2 receptors.[6] Older alpha-2 antagonists, such as tolazoline, have a lower affinity for the alpha-2 receptors[7] and are not as effective in completely reversing sedative effects of medetomidine; however, tolazoline is thought to mitigate alpha-2-induced cardiovascular and

respiratory adverse effects; however, no systematic data have been published to demonstrate that Vatinoxan, a newer antagonist, also shows promise for alleviating peripheral cardiovascular effects of alpha-2 agonists, without affecting the level of sedation. Vatinoxan does not penetrate the blood brain barrier, and therefore only acts on peripheral receptors.[8] Studies in sheep or dogs sedated with dexmedetomidine showed that vatinoxan had no apparent effect on sedation levels due to its strictly peripheral action, but was able to alleviate negative cardiovascular effects of the alpha-2 agonist.[9-11] In captive markhors (*Capra falconeri heptneri*) immobilized with ketamine and medetomidine, the administration of vatinoxan reduced medetomidine-induced hypertension without affecting the quality of immobilization,[12] and in captive red deer (*Cervus elaphus*) immobilized with medetomidine, tiletamine and zolazepam, vatinoxan reversed medetomidine-induced bradycardia and hypertension.[13]

Due to the proven negative cardiovascular and respiratory effects of combinations such as BAM and NalMedA, and the widespread use of these types of drugs for free-ranging wildlife immobilization, there is a need to evaluate practical and simple methods for reducing adverse effects. If an effective dose of tolazoline and/or vatinoxan can be identified, an obvious application would be to administer it to the animal as soon as it is immobilized, thereby immediately improving its physiological state while staying immobilized. At the end of the processing, the animal would then be reversed with atipamezole and naltrexone as usually done. Administering an additional injection to an animal after immobilization is easy and always possible, and therefore likely to be adopted by a large number of veterinarians or biologists. If proven beneficial, the data from this study have the potential of improving animal welfare and safety for thousands of animals of multiple species immobilized with medetomidine based combinations across North America and beyond.

In a study using Rocky Mountain elk, we are evaluating whether tolazoline and vatinoxan can improve the cardiovascular and respiratory side effects of the drug combination butorphanol-azaperone-medetomidine (BAM). We are comparing the baseline physiological data collected from elk immobilized with BAM alone with data collected from the same elk when immobilized with BAM and receiving either tolazoline or vatinoxan 20 minutes after onset of immobilization. Data analysis is pending.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Investigating nutrition influences on the metabolome of bighorn sheep

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Mary Wood, Galen O'Shea, Terry Engle, Robert Garrott, Pauline Nol

Understanding the nutritional status of wild ungulates provides valuable information on underlying population performance, habitat quality, and population resilience in the face of other environmental factors. Current methods to assess nutritional status and underlying environmental interactions in wild ungulates are limited, often requiring subjective measurements such as palpation and body condition scoring. While these methods provide information on the fat and muscling of an animal, they cannot document underlying metabolic processes and how those may change based on environmental influences.

Metabolomics is a relatively new field of study focused on measuring substrates or products of metabolism (metabolites) that are influenced by both genetic and environmental factors.[1] Some examples of metabolites include sugars, lipids, alkaloids, phenols, amino acids and fatty acids. Studies focused on metabolomics in wildlife are limited; but there is great potential for this field to develop tools to improve understanding of the interaction between environmental factors and metabolic processes in free-ranging wildlife.[2] A major benefit of this approach is that analysis only requires blood, a commonly collected sample at captures, and analysis of samples offers quantitative and repeatable results.

We initiated a small pilot study to compare serum metabolic profiles of captive BHS fed at maintenance or below maintenance energy requirements. We assigned ten adult female bighorn sheep to two groups: treatment (maintenance nutrient requirements restricted to 90% of BHS requirements) or control (fed at maintenance nutrient requirements). Groups were matched based on their baseline weight and rump fat measurements. This study followed a longitudinal design. Control animals remained on their current diet of grass/alfalfa hay *ad libitum* and a pelleted high-energy supplement given at 1.1kg/sheep per day. In December, 2020 Treatment animals were given a formulated diet to achieve up to an 18 percent loss of body mass over three to four months. Starting in April, 2021, treatment animals were placed back on the same diet as controls and monitored for body weight gains through June, 2021. BHS were weighed and

sedated at the beginning of the study and then once per month thereafter through June, 2021 to obtain ultrasound measurements of fat, body condition scoring, fecal samples, and blood samples.

Serum samples will be prepared for nuclear magnetic resonance spectroscopy using existing protocols.[3] All NMR spectra will be analyzed at 25°C on a Bruker 600 MHz (^{1}H Larmor frequency) AVANCE III solution spectrometer at the MSU NMR Center in Bozeman, MT. Statistical analyses will be conducted in R (R Core Team 2017) and MetaboAnalyst 3.0 and may include metabolomics pathway analysis, partial least squares discriminant analysis, random forests and boosting.

The animal handling and initial sample preparation for the study was complete in June 2021. In the coming year, we will compare metabolites in both groups to determine whether there are baseline differences in their metabolome and which metabolites account for those differences. Data may subsequently be compared to existing free-ranging BHS metabolite data. Through this approach, we can investigate the bighorn sheep metabolome for metabolites that might be associated with different nutritional states, particularly those that may be associated with catabolism of fat seen during the winter when sheep are on a lower quality diet and rely on fat reserves for survival.

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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 2020–30 June 2021

Principal Investigators: Dan Tripp

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 requires frequent maintenance and is often in need of repair limiting efficiency of field distribution. Improved bait distribution equipment that is more robust and reliable than the current model is needed to gain efficiency and reduce the cost of vaccine distribution. CPW has also developed a fleet of High Pressure Air (HPA) dusters[10-11] that improve work quality and efficiency of insecticide application to prairie dog burrows to control fleas. However, this design requires the use of high-pressure compressed air, which is cumbersome to transport and time consuming to maintain. A low-pressure compressor and air supply tank system to convert the

current High Pressure Air (HPA) dusters to a more efficient low-pressure system is needed to gain efficiency and reduce cost of insecticide application.

This project aims to design and build improved bait distribution equipment that is more robust and reliable than the currently available model. We will also design and build a low-pressure compressor and air supply system to convert the current High Pressure Air (HPA) dusters to a more efficient low-pressure system. These improvements will increase the safety, efficiency, and quality of plague management while reducing labor costs associated with plague management.

In the past year, we have designed and constructed six low-pressure, ATV mountable dusters (Fig. 1). These six units were field tested at Soapstone Prairie Natural Area in 2021. Fort Collins Natural Areas staff dusted over 28,500 burrows and the mean amount of dust dispensed was 6.3 grams in each burrow (Fig. 2). This initial field test demonstrated the utility of the design and revealed needed improvements. We learned that in temperatures above ~90°F a more robust electrical lead and fuse system is needed. We will retrofit the existing units to eliminate excessive fuse trips and will incorporate this modification into six new units we will build for work in the summer of 2021.

In 2020, we also preformed needed maintenance and repairers on the bait distribution equipment and we were able to complete all scheduled bait distribution. We have worked with two separate engineering and design companies in the last year that have been unable to achieve our goals toward development of improved bait distribution equipment. We have however, recently consulted with an engineer with ArduPilot and we have developed an initial plan to begin the redesign and construction of new bait distribution equipment.

Figure 1. A low-pressure duster mounted on an ATV. The first six prototypes were field tested at Soapstone Prairie Natural Area in 2021.

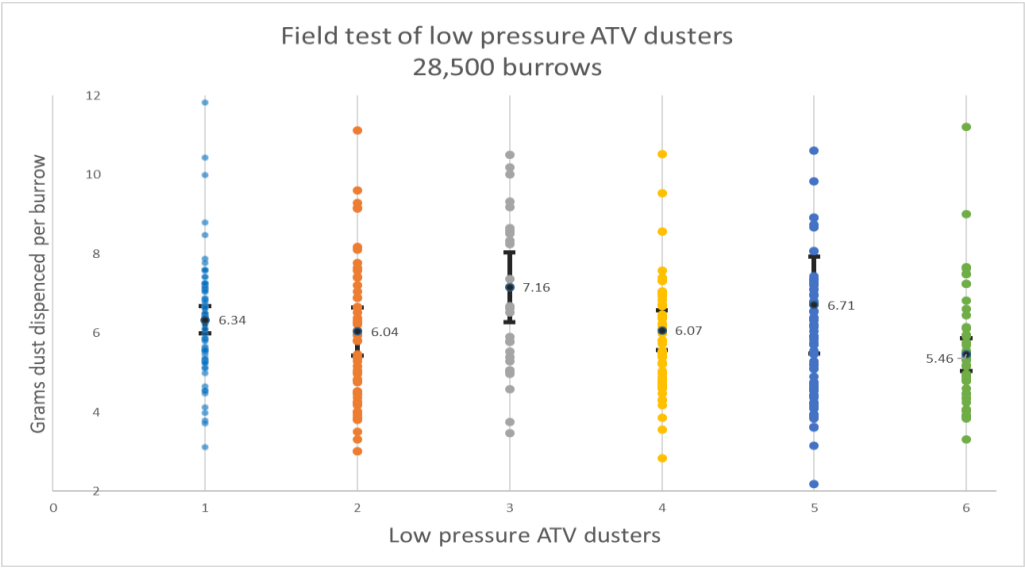

Figure 2. We field tested six low-pressure ATV dusters at Soapstone Prairie Natural Area in 2021. The mean grams dust dispensed per burrow for each of the six units is shown. Error bars are 95% confidence intervals.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Evaluation of sustained release and short acting haloperidol in captive bighorn sheep

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Kelsey M. Rayment, Lisa L. Wolfe, Mary E. Wood, Michael W. Miller

Several long-acting neuroleptics (LANs) have been used in wild animals to provide for sustained tranquilization without the need for repeated injections or treatments.[1] These drugs have been used for several decades to reduce handling stress, to improve the initial habituation period when transporting an animal into a novel environment, and to make approaching and sampling of animals possible with only minimal restraint.[1-3] The use of a chemotherapeutic agent that would reduce handling stress and potential injuries to animals and personnel can be an important adjunct to trap and transplant operations.

Haloperidol, in the butyrophenone group of LANs, acts as a tranquilizer and antipsychotic.[4] Effects are produced through a central dopaminergic and peripheral adrenergic blockade. Butyrophenones produce less sedation and fewer anticholinergic effects than other LANs but can have a higher incidence of extrapyramidal side-effects.[5] Although the duration of effect is not as long as seen with other LANs; haloperidol is the only LAN available in the US. Haloperidol has been delivered by intramuscular (IM) injection with good to excellent results in a number of African wildlife species including impala, springbok, duiker and steenbok.[3]

Haloperidol is available in the US in two compounded formulations.[1] Haloperidol, formulated as a lactate, is most readily available as either an injectable solution or oral powder (Wildlife Pharmaceuticals/ZooPharm, Laramie, Wyoming). Haloperidol decanoate is a longer acting "sustained release" formulation recently made available through Wildlife Pharmaceuticals (Windsor, Colorado). Both formulations have been used in North American wildlife, including bighorn sheep (*Ovis canadensis*), but the formulations are not interchangeable and problems may arise when haloperidol lactate is administered using dosing recommendations for haloperidol decanoate.[1] Data directly comparing the duration of action, pharmacodynamics, and behavioral side-effects of both formulations in bighorn sheep would be useful in field applications in

Colorado and elsewhere. Moreover, information comparing how these formulations behave will be useful for selecting the appropriate drug for a given capture and translocation project.

We utilized six captive adult ewes in a crossover study wherein three sheep were randomly assigned to receive the sustained release haloperidol and three to receive the standard haloperidol. The sheep were given one of the haloperidol formulations by hand injection intramuscularly in the hindquarters. Blood samples were collected from the jugular vein at 1, 4, 8, 12, 24, 48, and 72 hours after the haloperidol is administered in EDTA-vacutainer tubes. Blood samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Texas A&M Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, Texas, USA) after isolation by Solid Phase Extraction (SPE). Behavioral observations were recorded before each blood draw and prior to haloperidol administration. We collected observations of confinement tolerance, ease of handling for blood draw, appetite and any side effects such as akathisia, akinesia, dyskinesia, tremors, and ataxia.

Results from the first portion of the crossover suggest that, when dosed similarly, peak plasma concentrations of haloperidol lactate are much higher than haloperidol decanoate though duration of detectable plasma concentrations were similar (Fig 1). Unfortunately, there were problems with sample analysis in the second portion of the study and the laboratory was unable to account for substantial analysis error. We are currently in the process of identifying a new laboratory to analyze samples and complete the study.

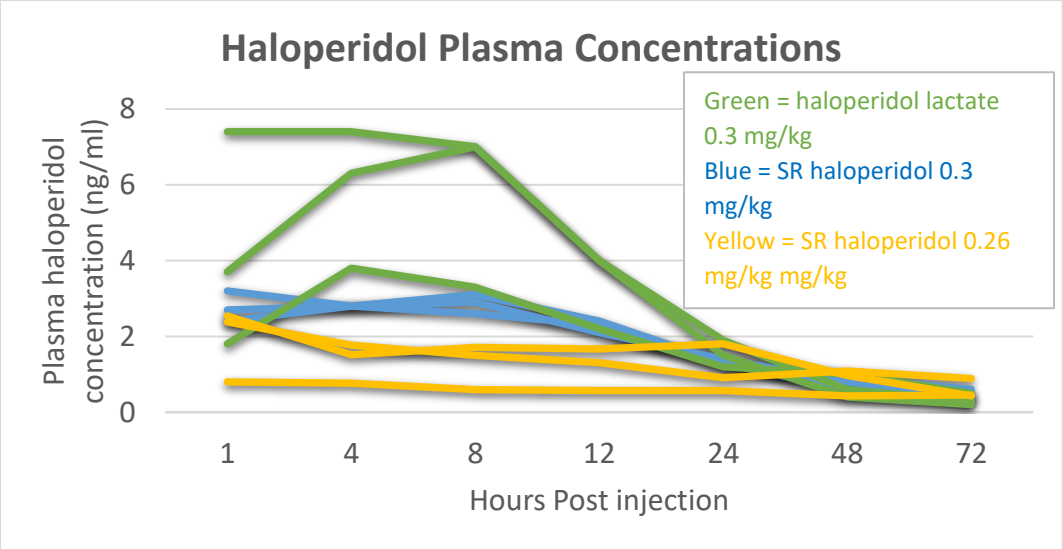

Figure 1: Plasma concentrations of haloperidol at different formulations/doses

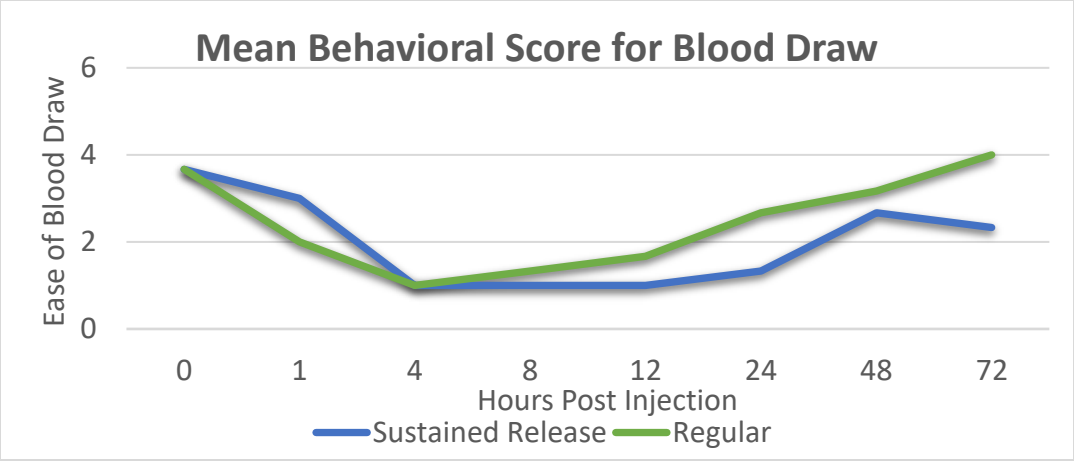

Figure 2: Mean behavioral score for ease of blood draw. 1=very easy, 5=unable to draw

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Investigation of diarrhea in mountain goats on Mount Evans (G4)

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Karen A. Fox, Lance Carpenter

In the late summer of 2013 (end of August), the United States Forest Service noticed a mountain goat that had black on the back of its legs. They again saw this same mountain goat, took pictures, and alerted CPW. Based on the photograph, it was thought to be Johne's disease, a known cause of diarrhea in the bighorn sheep population on Mount Evans. CPW euthanized two mountain goats (1 yearling and 1 kid) in late August/early September for a necropsy. The necropsies results indicated that the mountain goats did not have Johne's but were diagnosed with bacterial enteritis and high loads of *E. coli*. This was the first time (to our knowledge) that diarrhea had been observed in mountain goats in the Mount Evans area in the fall. CPW monitored the mountain goats from the end of August through October and marked the sick animals with oil-based paintballs. It was determined that diarrhea was mainly affecting kids and yearlings. CPW observed 28 symptomatic mountain goats throughout G4, of which 17 were marked. During the marking of sick mountain goats, only two marked animals were resighted. There was uncertainty if this potential disease outbreak would affect the survivorship of the sick kids and yearlings. In 2014, after the summer bighorn sheep and mountain goat survey, it was determined that almost an entire age class of mountain goats were lost (Figure 1) in 2014.

Colorado Parks and Wildlife observed occasional sick kids and yearlings from 2014-2018 (n=8). The kids and yearlings had the same symptoms as observed in 2013. For the most part, sick mountain goats observed were not seen again. In 2018, one ill kid was euthanized and taken for a necropsy. The necropsy results were similar to the results of the necropsied mountain goats in 2013. Based on the summer bighorn sheep and mountain goat surveys (2015-2019), CPW did not see marked declines in the kid and yearling:adult ratios (kid:adult range=31-35; yearling:adult range= 27-30) that was documented in 2014. In 2019, another severe diarrhea outbreak occurred. In October, seven kids were observed with severe diarrhea, and two were euthanized for a necropsy. The necropsy results were similar to the other G4 necropsies,

although several findings could not be attributed solely to infection with *E. coli*, and additional testing was pursued. In January 2020, a sick juvenile mountain goat was removed from the G15 herd, with similar findings to the goats from G4. This raised concerns about the spread of the disease beyond the G4 herd. In 2020, after the summer bighorn sheep and mountain goat survey, it was determined that almost an entire age class of mountain goats were lost (Figure 2), similar to 2014. In 2019, extensive disease testing was performed and several pathogens were identified, but a definitive primary cause for diarrhea in these mountain goats was not identified.

In 2020, we initiated a pilot study to understand the importance of bacteria such as *E. coli*, and other pathogens contributing to severe diarrhea in G4. Fecal samples from marked kids (n=14), their nannies (n=14), and yearlings (n=2) were collected monthly as opportunistically available from July – October for a total of 60 samples. The results from these samples do indicate that there are fluctuations in *E. coli* seasonally, with the heaviest loads of *E. coli* detected during July and August, lower loads in September, and almost no detections of *E. coli* in October (Figure 3). This could suggest that higher loads are present at times that human activity is highest in the area. These trends are present in both the adult and immature mountain goats in G4, suggesting it is not just a function of rumen maturation (although *E. coli* loads were higher in kids than adults). Other bacteria and parasite results somewhat mirrored the *E. coli* results, suggesting that the microflora of the gut was generally altered and out of balance from normal. For example, *Clostridium pefringens* Type A (a bacteria that is often encountered as a nonspecific indicator of gut flora imbalance) was identified only in kids in July and August with high *E. coli* loads. It was not detected in any adult samples). No samples were positive for Johne's disease, and no viruses were detected by electron microscopy. A primary cause for diarrhea in these mountain goats remains uncertain. To better understand the importance of bacteria such as *E. coli*, and to further investigate the roles of other pathogens, continued robust sampling of the population is warranted.

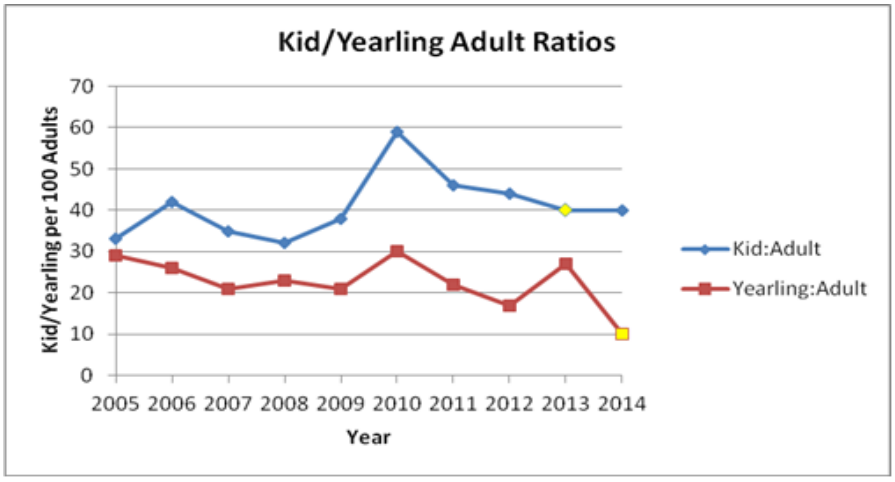

Figure 1. Kid/yearling adult ratios from the Mount Evans summer ground surveys 2005-2014. The yellow squares denote the kids' diarrhea outbreak in 2013 with a sharp decline in the yearling/adult ratio

Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Sinusitis and conjunctivitis in a wild turkey associated with Avibacterium-like bacteria

Period Covered: 1 July 2020–30 June 2021

Principal Investigators: Jayne Ellis, Christopher Anderson, Kerry Sondgeroth, Karen A. Fox

In January of 2020, Colorado Parks and Wildlife (CPW) was contacted about sick and dying wild turkeys (*Meleagris gallopavo*) in Pueblo County, Colorado. Necropsy of one affected turkey revealed severe catarrhal and fibrinous sinusitis and conjunctivitis (Figure 1), and culture identified bacteria most consistent with *Avibacterium paragallinarum*, the cause of infectious coryza in domestic chickens. However, confirmation of species by sequencing of the 16S rRNA gene showed less than 96% homology, suggesting a novel *Pasteurellaceae* bacteria.

The objective of this study is to characterize the bacteria associated with disease, and determine the extent of the disease within the flock, general area, and distant to the flock within the state of Colorado. Eight additional wild turkeys from the affected flock (~ 25% of the flock) were culled for disease surveillance. Gross necropsy and histologic evaluation of the nasal and infraorbital sinuses was performed on all birds, along with aerobic culture, and PCR for *Mycoplasma synoviae*, *Mycoplasma gallisepticum,* and *Mycoplasma meleadgridis*. Choanal swabs were also collected from 17 live domestic pigeons located on the property where the sick turkey was originally identified, as well as 9 live wild turkeys located near the flock but considered a separate, unconnected population. Finally, 7 wild turkeys were culled from a flock near Pagosa Springs (far distant to the affected flock near Pueblo), and tested as described above for the 8 culled birds in the Pueblo flock.

Infraorbital sinus swabs from four of the eight culled wild turkeys in the affected flock showed heavy growth of *Pasteurellaceae* bacteria and had mild hyperplastic and lymphoplasmacytic sinusitis. MALDI-TOF analysis of the bacterial isolates was most consistent with an *Avibacterium* species, most closely resembling *A. gallinarum*. 16S gene sequencing demonstrated greater than 98% homology between the isolates from the Pueblo flock, and less than 96% identity to other *Pasteurellaceae*. Full genome sequencing provided similar results, with less than 87% homology to other *Pasteurellaceae,* and greater than 97% homology to each

other. These findings continue to suggest that a novel species of *Pasteurellaceae* is present within the Pueblo flock with at least one turkey showing severe clinical signs of disease.

One of the 17 sampled domestic pigeons provided a *Pasteurellaceae*-type bacteria on culture, as did 8 of the 9 sampled live turkeys near Pueblo but distant to the affected flock. None of the 7 wild turkeys sampled near Pagosa Springs provided any *Pasteurellaceae*-type bacteria on culture. Sequencing data from the live domestic pigeons and wild turkeys is pending. Our results identify severe sinusitis and conjunctivitis in a wild turkey, associated with presence of an *Avibacterium*-like bacteria. The presence of this bacteria in other birds in the flock, in the absence of significant disease, suggests other factors may be involved in development of severe conjunctivitis and sinusitis. The extent of the bacteria within the state of Colorado is still to be determined.

Figure 1. Wild turkey with eyelid removed to show mat of fibrin covering eye. The infection extended into the upper respiratory sinuses of the head and can be seen filling the infraorbital sinus rostral to the eye in this image.

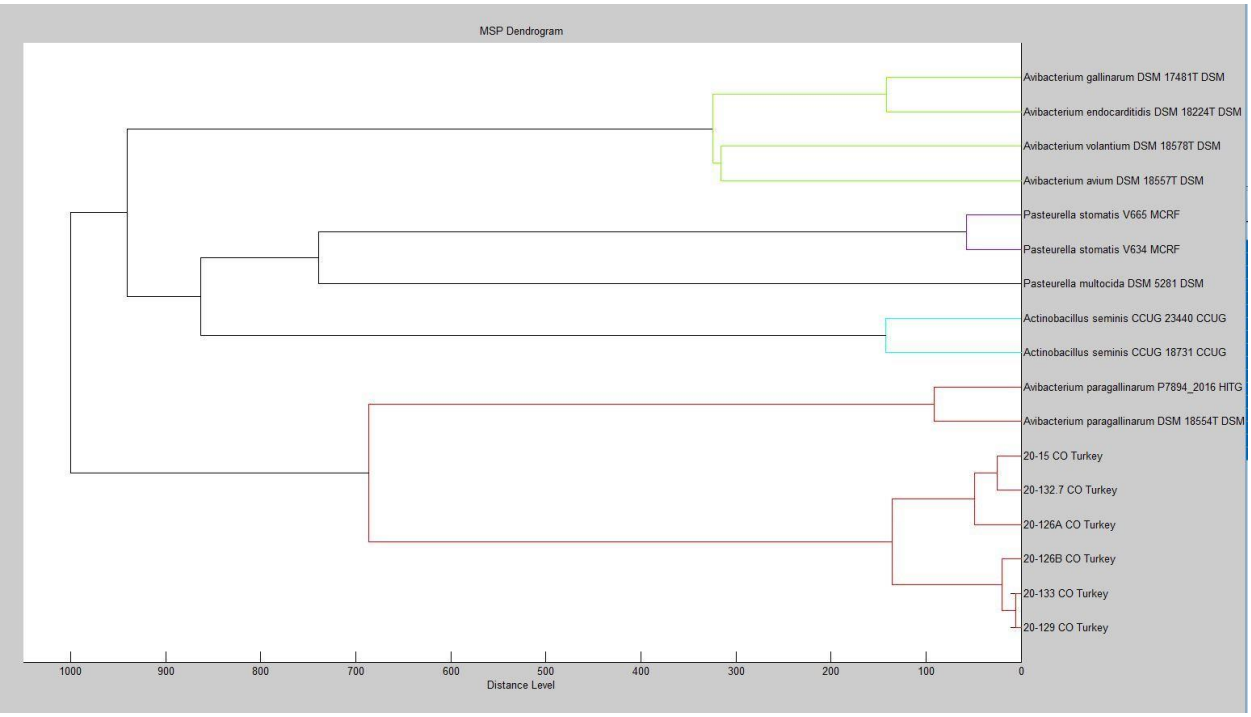

Figure 2. Dendrogram of wild turkey *Avibacterium*-like isolates as compared to other known, similar *Pasteurellaceae*. The lowest cluster of organisms represents the Colorado turkey isolates. The closest similarity is to *Avibacterium gallinarum*, with less than 87% homology.

SUPPORT SERVICES

Veterinary medical support provided to Colorado Parks & Wildlife during 2020-2021.

Location of services & primary user	Species	Type of veterinary medical support
CPW Foothills Wildlife Research Facility (FWRF); Maicie Sykes & researchers	mule deer, elk, bighorn sheep, pronghorn, bobcat, prairie dogs, mallard ducks, 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; C. Anderson, M. Alldredge, E. Bergman, N. Rayl & collaborators	mule deer, elk	Ultrasounded, sampled, & assessed body condition of mule deer and elk as part of various field research studies throughout southern and western Colorado. Provided tailored sedation as well as supportive veterinary care for captured animals.
Statewide; district & area wildlife managers, terrestrial biologists	bighorn sheep	Provided veterinary care and logistical support for capture efforts. Provided materials, support and training for collection and processing of genetics and disease samples.
Statewide; district & area wildlife managers, terrestrial biologists	mountain quail	Collection and processing of disease samples and animal holding for translocation effort.
Statewide; district & area 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, provide training on proper drug & equipment use.
Field assistance with other CPW research & management projects	moose, bighorn sheep, mountain goat, mountain lion, mule deer, elk, prairie dog, turkey, greater sage grouse, wolf etc.	Provided capture assistance, equipment, field sampling, medical supplies & assistance, training, & other forms of support for various monitoring, translocation, & research projects statewide.

Training and education support provided during 2020-2021.

Location of services & primary user	Species	Type of training support
CPW Foothills Wildlife Research Facility (FWRF); Statewide; district & area wildlife managers, terrestrial biologists	Native Colorado Mammals	A wildlife capture and handling training class was provided for District Wildlife Manager trainees and smaller introductory classes were offered for students or field technicians. Capture classes included lectures on drug use regulations and recordkeeping, pharmacology of capture drugs, dosing, safety and types of equipment. These classes also included hands on capture and handling of animals at FWRF. A new course was provided for biologists to refresh knowledge and technical skills and present advanced supportive care techniques. *In situ* training was provided at meetings and on-site during various capture operations statewide.
Statewide; district & area wildlife managers, terrestrial biologists	Native Colorado Mammals	Dart projector calibration clinics and equipment consultation were provided to help maintain high quality capture drug delivery from remote delivery devices. Calibration clinics provide training for proper equipment use and maintenance and ensure proper performance of drug delivery equipment.
CPW FWRF, 4th year veterinary students	Native Colorado Mammals	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; district & area wildlife managers, terrestrial biologists	Native Colorado Cervids	Four on-site trainings and one virtual training were provided on chronic wasting disease sampling and data collection.
FWRF; Statewide; district & area wildlife managers	Native Colorado Mammals	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	Native Colorado Mammals	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	Plague management, bait distribution equipment and duster operation training and maintenance.
Statewide, Park Rangers	Native Colorado Mammals	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 support provided during 2020-2021

Location of services & primary user	Species	Type of support
Statewide; species conservation and terrestrial biologists, CPW researchers	Gunnison's and white-tailed prairie dogs, black footed ferrets	Plague vaccine bait manufacture. Produced ~340,000 baits to vaccinate ~7,500 acres
South Park Individual Population Area, conservation and terrestrial Biologists	Gunnison's prairie dogs	Vector Control for Plague Management: ~380 acres dusted
Statewide; Terrestrial Resources Program, terrestrial biologists	Elk	Informally analyzed & summarized data showing the relationship between CWD prevalence in elk and mule deer harvested in the same GMU; developed recommendations for 2021 elk monitoring that will lower statewide programmatic costs by sampling specific hunt codes rather than entire Data Analysis Units over all seasons.
Statewide; Terrestrial Resources Program, terrestrial biologists	Mule deer, white-tailed deer	Informally examined within-year CWD prevalence trends across hunting seasons, as well as prevalence among mule deer vs. white-tailed deer within the same geographic area; initial work suggests more formal analyses could help inform future disease management approaches.
Statewide; Terrestrial Resources Program, terrestrial biologists	Mule deer	Informally examined relationships between CWD prevalence and adult female mule deer survival; initial work suggests more formal analyses could help inform future population modeling and assessment of disease impacts.
Statewide; Terrestrial Resources Program, terrestrial biologists	Mule deer, white-tailed deer, elk	Summarized statewide chronic wasting disease (CWD) surveillance data; updated prevalence tables & maps.
Statewide; Terrestrial Resources Program, terrestrial biologists	Native Colorado mammals	Developed guidance for wildlife rehabilitation in coordination with the Colorado Department of Public Health and the Environment.

Laboratory and Diagnostic Support Provided during 2020-2021

Location of services & primary user	Species	Type of support
CPW Foothills Wildlife Research Facility (FWRF); Statewide; district & area wildlife managers, terrestrial biologists	Mule deer, white-tailed deer, elk, moose	Chronic wasting disease surveillance and monitoring. Facilitated testing of over 7,500 samples for CWD
FWRF; Statewide; district & area 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
FWRF; Statewide; district & area wildlife managers, terrestrial biologists, researchers	Cottontails, jack rabbits, snowshoe hare	RHDV2 testing of tissue and fecal samples
FWRF; Statewide; district & area wildlife managers, terrestrial biologists, researchers	Coyotes, wolves	Echinococcus multi-plex testing on fecal samples
FWRF; Statewide; district & area wildlife managers, terrestrial biologists, researchers	Native Colorado Wildlife	Serum and tissue banking. 537 samples added to existing serum and tissue banks.
CPW researchers and CPW animal care and use committee	mule deer, elk, moose, bighorn sheep, various avian species	Provided necropsy services to determine cause specific mortality for various ongoing research projects. Provided necropsy services for capture-related mortalities to help assess capture methods for CPW animal care and use committee.

2020 CPW Wildlife Health Program Mortality Report
(Submissions from Jan - Dec 2020)

Species (#nx cases)	Necropsy Findings	# affected	Notes
Avian			
16	Starvation/Exposure	100+	Summer snowstorm mortality: bluebirds, warblers, western wood pewees; 2 bluebirds from fall snowstorm
5	Bacterial/Botulism, Type C	30+	30+ mallards confirmed, various other ducks unconfirmed
9	Bacterial/Sinusitis, *Avibacterium paragallinarum*	9	1 wild turkey affected, 8 others in flock positive without clinical signs
1	Bacterial/Avian Tuberculosis	1	1 great horned owl
5	Trauma/Hit by Car	12	8 crossbills, 4 wild turkeys
5	Trauma/Domestic Dog Predation	5	5 wild turkeys
1	Truama/Gunshot	1	1 American white pelican
1	Trauma/Electrocution	1	1 bald eagle
1	Trauma/Various	1	1 Eurasian collared dove
3	Viral/West Nile	10+	8+ crows confirmed, 1 house sparrow, 1 raven unconfirmed
1	Viral/Avian Pox	1	1 wild turkey
3	Toxic/Unconfirmed	20+	20+ feral pigeons, bait found in crops but unconfirmed type of toxin
3	Toxic/Anticoagulant Rodenticide	3	3 barn owls, also bacterial and fungal pneumonia
3	Visceral Gout	4	4 Canada geese, unknown cause
2	Husbandry Related	2	2 mallards
Bat			
42	Disease Surveillance	42	Multiple species, all negative for SARS CoV-2
8	Disease Surveillance	8	Multiple species, all negative for white nose syndrome

Species (#nx cases)	Necropsy Findings	# affected	Notes
15	Starvation/Exposure	15	12 little brown bats (juvenile, stranded from roosts)
2	Viral/Rabies	2	1 big brown bat, 1 hoary bat
1	Undetermined	1	1 big brown bat, nondiagnostic sample
1	Trauma/Undetermined Cause	1	1 silver-haired bat
1	Apparently healthy	1	1 long-eared myotis
Bear			
7	Human Conflict	7	Trash/human food in stomach (n=4), concurrent cancer (n=1)
5	Toxin/Rodenticide	5	5 anticoagulant, 1 bromethalin
1	Toxin/Antifreeze	1	
2	Illegal Take	2	
1	Acquired storage disease	1	Lipofuscinosis, blind
Beaver			
1	Trauma/Bite Wounds	3	Suspect domestic dog predation
Bighorn Sheep			
15	Disease Surveillance/Hit by Car	15	3 sinus tumor, 5 leukotoxigenic *Pasteurellaceae* , 3 *Mycoplasma ovipneumoniae, 2 Pasteurella multocida*
2	Disease Surveillance/Hunter Harvest	2	
2	Disease Surveillance/Live Animals	2	1 leukotoxigenic *Pasteurellaceae , 1 Pasteurella multocida*
1	Disease Surveillance/Starvation	1	1 leukotoxigenic *Pasteurellaceae*
1	Disease Surveillance/Predation	1	
1	Disease Surveillance/Capture Mortality	1	

Species (#nx cases)	Necropsy Findings	# affected	Notes
Chipmunk			
1	Capture Related	1	
Cottontail rabbit			
13	Viral/Rabbit hemorrhagic disease	100+	
2	Viral/Undetermined	2	Suspect viral, negative for multiple viral etiologies
11	Predation/Domestic Dog or Cat	11	
5	Bacterial/Tularemia	6	
1	Bacterial/Undetermined	1	Suspect bacterial, negative for multiple bacterial etiologies
6	Starvation/Orphaned	6	
3	Parasitic/Coccidiosis	3	
2	Malnutrition/Winter Mortality	2	Also fungal and bacterial pneumonia
1	Trauma/Blunt trauma	1	
1	Trauma/Gunshot	1	
1	Trauma/Stuck in fence	1	
9	Undetermined	9	Advanced autolysis
Domestic Animals			
2	Viral/Rabbit hemorrhagic disease	20+	Feral domestic rabbits found dead, remaining animals in the population were culled
5	Cull due to contact with bighorns	5	5 domestic goats, 1 case with caseous lymphadenitis, leukotoxigenic Pasteurellaceae, and *Bibersteinia trehalosi*
1	Suspected depredation	1	1 domestic bovine calf (determined to be stillborn vs depredation)

Species (#nx cases)	Necropsy Findings	# affected	Notes
Elk			
0	Chronic Wasting Disease	28	Includes all CWD submissions from 2020-2021
1	Clinical CWD	1	
10	Starvation/Orphaned calf	10	
3	Starvation/Tooth wear	3	One case also had CWD
1	Malnutrition/Winter mortality	1	
4	Bacterial/Necrobacillosis	4	Two with oral lesions from plant thorns (live animal biopsies)
2	Bacterial/Pasteurellosis	2	
1	Bacterial/Colibacillosis	1	Calf, also had giardia
1	Bacterial/Lung Abscesses	1	
2	Encephalitis/Undetermined	2	
1	Parasitic/Elaeophorosis	1	
1	Stillborn	1	No apparent underlying disease
1	Upper airway obstruction	1	Calf
1	Trauma/Blunt trauma	1	Calf, unknown cause
1	Trauma/Leg fracture	1	Chronic bone callus submitted
1	Predation/Mountain Lion	1	
1	Viral/Fibromatosis	1	
1	Traumatic Reticuloperitonitis (Hardware Disease)	1	Also had CWD
1	Illegal Take	1	

Species (#nx cases)	Necropsy Findings	# affected	Notes
1	Normal	1	Normal serous blood clot submitted as possible mass
3	Capture related	3	
4	Undetermined	4	Tissues only submitted
Fox			
1	Viral/Canine distemper virus	1	1 gray fox
1	Parasitic/Sarcoptic mange	1	
1	Trauma/Unknown	1	
1	Renal failure	1	Cause undetermined
2	Undetermined	2	
Fox Squirrel			
4	Toxin/Rodenticide	4	3 bromethalin poisoning, 1 cholecalciferol poisoning
2	Bacterial/Plague	2	
Jackrabbit			
6	Viral/Rabbit Hemorrhagic Disease	9	
Kangaroo Rat			
1	Undetermined	1	
Marmot			
1	Undetermined	1	Marked autolysis, rabies negative
Mink			
1	Viral/Canine Distemper	1	

Species (#nx cases)	Necropsy Findings	# affected	Notes
Moose			
2	Parasitic/Elaeophorosis	2	
1	Parasitic/Winter ticks	1	
1	Lymphadenitis	1	Botryomocosis, *Mannheimia* species
1	Bacterial/Necrobacillosis	1	With caval syndrome
1	Undetermined	1	Tissues only submitted
Mountain Goat			
1	Diarrhea	1	Unknown cause, ongoing research project
Mountain Lion			
6	Human Conflict	6	4 juvenile, 1 subadult, 1 adult
1	Domestic Dog Attack	1	1 adult
1	Bacterial/Plague	1	
1	Suspect illegal take	1	Age determination
1	Capture Related	1	
1	Starvation	1	1 subadult male
1	Trauma/Bite Wounds	1	1 juvenile, suspect domestic dog predation
1	Neoplasia	1	Cholangiocarcinoma
1	Euthanized/Declining condition	1	
Mouse			

Species (#nx cases)	Necropsy Findings	# affected	Notes
1	Undetermined	10+	Found dead alongside rabbits with RHDV mortality event (grasshopper mice)
2	Undetermined	2	1 deer mouse, 1 unknown species
Mule Deer			
1	Chronic Wasting Disease	272	Includes all CWD submissions from 2020-2021
1	CWD/Clinical Cases	1	
6	Malnutrition/Winter Conditions	6	1 aged adult, with heavy parasitism and neoplasia (colorectal carcinoma); 5 juvenile, also with parasitism
2	Starvation/Orphaned	2	
1	Dietary/Rumen Acidosis	1	
6	Viral/Adenoviral hemorrhagic disease	6	3 fawns, 3 adults; 1 also had pasteurellosis
1	Viral/Bovine Viral Diarrhea	1	Also had MCF
	Viral/Malignant Catarrhal Fever	1	Also had BVD
1	Viral/Epizootic Hemorrhagic Disease	1	EHDV-2
1	Viral/Bluetongue	1	BTV-2, BTV-11
3	Bacterial/Conjunctivitis	3	
1	Bacterial/Brain abscess	1	
1	Bacterial/Colibacillosis	1	
1	Bacterial/Endometritis	1	*Truperella pyogenes, Fusobacterium necrophorum*
1	Bacterial/Bronchopneumonia	1	Too autolyzed for culture
3	Stillborn/Congenital Abnormalities	3	Contracted limbs (n=3), cleft palate (n=2), brachygnathia inferior (n=2)
1	Stillborn/Premature	1	
1	Euthanized/Congenital Abnormalities	1	Anterior segment dysgenesis with micropthalmia

Species (#nx cases)	Necropsy Findings	# affected	Notes
2	Trauma/Undetermined	2	
1	Trauma/Skull Fracture at Pedicle	1	1 yearling male
1	Trauma/Hit by Car	1	
2	Failed Predation/Unknown Species	2	
1	Predation/Bear	1	
1	Predation/Domestic Dog	1	Infected bite wounds
2	Human Conflict	2	2 yearling males, 1 with history of illegal feeding
1	Legal/Suspect Poaching	1	
3	Undetermined	2	Tissues only submitted
Prairie Dog			
2	Bacterial/Plague	2	
1	Predation/Avian	1	
1	Undetermined/Scavenging	1	
Pronghorn			
1	Bacterial/Necrobacillosis	1	Tooth root abscess
1	Undetermined	1	Gunshot rule out
1	Undetermined	1	Tissues only submitted
Raccoon			
8	Viral/Canine Distemper	10	
1	Toxicity/Rodenticide	1	Bromethalin
1	Undetermined	1	

Species (#nx cases)	Necropsy Findings	# affected	Notes
Snowshoe hare			
1	Trauma/Hit by Car	1	
Skunk			
2	Rabies	2	
1	Trauma/Undetermined	1	Suspect domestic dog predation
Wolf			
6	Disease Surveillance/Feces	3	*Echinococcus canadensis* (n=3)
Wood Rat			
1	Pneumonia/Undetermined	1	Granulomatous pneumonia
White-tailed Deer			
0	Chronic wasting disease	19	Includes all CWD submissions from 2020-2021
1	CWD/Clinical cases	1	
1	Brain abscess	1	
1	Undetermined	1	Tissues only submitted

APPENDIX A: Wildlife Health Research Publication Abstracts

A Prototype Compressed Air Insecticide Applicator and Quality Control Monitoring for Plague Management on Prairie Dog Colonies

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Citation: Tripp DW, Emslie AC, Sack DA & Zieschang M. 2021. A prototype compressed air insecticide applicator and quality control monitoring for plague management on prairie dog colonies. Wildlife Society Bulletin. 45:176-183. https://doi.org/10.1002/wsb.1165

ABSTRACT: Plague causes declines in native wildlife populations including those of prairie dogs (*Cynomys* sp.) and endangered black-footed ferrets (*Mustela nigripes*). The infusion of insecticides into prairie dog burrows is a vector control method regularly used to suppress or prevent plague transmission by fleas. Detailed methods on how insecticide application equipment (dusters) are calibrated or how the quality of application is monitored are not regularly reported. We describe a new prototype duster and compare its performance to commercially available equipment using quality control monitoring procedures. We infused 0.05% deltamethrin (dust) into burrows of Gunnison's and black-tailed prairie dogs (*C. gunnisoni*, *C. ludovicianus*) in Colorado in 2014–2017. The prototype and commercial dusters distributed a similar amount of dust ($\bar{x}$ = 5.39 and 5.29 g/burrow P = 0.4). However, the prototype dusters required less operation time to dispense dust than the commercial dusters ($\bar{x}$ = 1.0 sec vs. 7.69 sec/burrow, $P \leq 0.0001$). If we applied a standard operating time of 5 sec to all commercially available units, 60% (20 of 34) would fail to dispense ≥4 g of dust. The prototype dusters were more consistent in their dust output with all units (12 of 12) dispensing ≥4 g of dust with an operation time of only 1 sec. Controlled and accurate application of insecticide is difficult to achieve if quality control monitoring and equipment calibration are not conducted. The efficacy of vector control is likely only as effective as the quality and precision of the application of the insecticide itself. When evaluating plague management outcomes it is important to consider if some observed limitations of efficacy are caused by the failure of the management tool or the failure of managers to effectively use the tool. Future work to calibrate the prototype dusters to dispense larger target doses and performance testing with other insecticide formulations is needed. Additional modifications to adapt the prototype dusters for use on ATV-mounted platforms may increase the utility of the equipment.

Detection of chronic wasting disease in mule and white-tailed deer by RT-QuIC analysis of outer ear

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Citation: Ferreira, N.C., Charco, J.M., Plagenz, J., Orru, C.D., Denkers, N.D., Metrick, M.A., Hughson, A.G., Griffin, K.A., Race, B., Hoover, E.A. and Castilla, J., 2021. Detection of chronic wasting disease in mule and white-tailed deer by RT-QuIC analysis of outer ear. Scientific Reports, 11(1), pp.1-9.

ABSTRACT: Efforts to contain the spread of chronic wasting disease (CWD), a fatal, contagious prion disease of cervids, would be aided by the availability of additional diagnostic tools. RT-QuIC assays allow ultrasensitive detection of prion seeds in a wide variety of cervid tissues, fluids and excreta. The best documented *antemortem* diagnostic test involving RT-QuIC analysis targets lymphoid tissue in rectal biopsies. Here we have tested a more easily accessed specimen, ear pinna punches, using an improved RT-QuIC assay involving iron oxide magnetic extraction to detect CWD infections in asymptomatic mule and white-tailed deer. Comparison of multiple parts of the ear pinna indicated that a central punch spanning the auricular nerve provided the most consistent detection of CWD infection. When compared to results obtained from gold-standard retropharyngeal lymph node specimens, our RT-QuIC analyses of ear samples provided apparent diagnostic sensitivity (81%) and specificity (91%) that rivaled, or improved upon, those observed in previous analyses of rectal biopsies using RT-QuIC. These results provide evidence that RT-QuIC analysis of ear pinna punches may be a useful approach to detecting CWD infections in cervids.

Opportunistic surveillance of captive and free-ranging bighorn sheep (Ovis canadensis) in Colorado, USA, for transmissible spongiform encephalopathies

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Citation: Fox, K.A., Muller, S.M., Spraker, T.R., Wood, M.E. and Miller, M.W., 2021. Opportunistic surveillance of captive and free-ranging bighorn sheep (Ovis canadensis) in Colorado, USA, for transmissible spongiform encephalopathies. The Journal of Wildlife Diseases, *57*(2), pp.338-344.

ABSTRACT Bighorn sheep (*Ovis canadensis*) are predicted to have a degree of susceptibility to the transmissible spongiform encephalopathies (TSE) chronic wasting disease and scrapie. We opportunistically screened 127 captive bighorn sheep and 152 free-ranging bighorn sheep in Colorado, US for the presence of TSE over a period of 35 yr. None of the animals demonstrated clinical signs, gross pathology, histopathology, or immunohistochemical staining patterns suggestive of TSE.

Hunting pressure modulates prion infection risk in mule deer herds

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2 These authors contributed equally to this study.

Citation: Miller, M.W., Runge, J.P., Holland, A.A. and Eckert, M.D., 2020. Hunting pressure modulates prion infection risk in mule deer herds. Journal of Wildlife Diseases, 56(4), pp.781-790.

ABSTRACT The emergence of chronic wasting disease, an infectious prion disease of multiple deer species, has motivated international calls for sustainable, socially accepted control measures. Here, we describe long-term, spatially replicated relationships in Colorado, US, mule deer (Odocoileus hemionus) herds that show hunting pressure can modulate apparent epidemic dynamics as reflected by prevalence trends. Across 12 areas in Colorado studied between 200218, those with the largest declines in annual hunting license numbers (pressure) showed the largest increases in the proportion of infected adult (2–yr-old) male deer killed by hunters (prevalence); prevalence trends were comparatively flat in most areas where license numbers had been maintained or increased. The mean number of licenses issued in the 2 yr prior best explained observed patterns: increasing licenses lowered subsequent risk of harvesting an infected deer, and decreasing licenses increased that risk. Our findings suggest that harvesting mule deer with sufficient hunting pressure might control chronic wasting disease—especially when prevalence is low—but that harvest prescriptions promoting an abundance of mature male deer contribute to the exponential growth of epidemics. Key words: Chronic wasting disease, control, epidemiology, hunting, mule deer, Odocoileus hemionus, prion, risk.

Effect of oral copper supplementation on susceptibility in white-tailed deer (*Odocoileus virginianus)* to chronic wasting disease

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Citation: Wolfe, L.L., Conner, M.M. and Miller, M.W., 2020. Effect of oral copper supplementation on susceptibility in white-tailed deer (Odocoileus virginianus) to chronic wasting disease. *Journal of wildlife diseases*, *56*(3), pp.568-575.

ABSTRACT: Chronic wasting disease (CWD) is an infectious disease, but reported associations suggest several metals—especially copper (Cu) and manganese—potentially play a role in this and other prion diseases. To assess the utility of dietary Cu supplementation in protecting white-tailed deer (Odocoileus virginianus) from CWD, we compared incidence and disease course among individuals naturally exposed to CWD while being maintained on sustained-release Cu boluses or unsupplemented (control). Oral Cu supplementation increased liver tissue Cu concentrations compared to controls but did not affect susceptibility to CWD or survival after natural exposure in the captive white-tailed deer we studied. Over the 27 mo study, 89% (8/9) of the Cu-supplemented deer and 86% (6/7) of control deer became CWD-infected. Survival to 27 mo post exposure did not differ between Cu-supplemented and control deer: model-averaged survival probabilities to 27 mo were 0.45–0.47 for all combinations of Cu treatment and

PRNP gene haplotype presence. The PRNP gene haplotype influenced the probability of deer remaining biopsy negative for at least 17 mo but did not affect overall susceptibility.

Tissue Residue Levels of the Tranquilizer Combination of Butorphanol, Azaperone, and Medetomidine, and the Antagonists, Naltrexone, Atipamezole, and Tolazoline, in Black Bears (Ursus americanus) Post immobilization

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Citation: Wolfe, L.L., Mays, T., Fisher, M.C. and Miller, M.W., 2020. Tissue Residue Levels of the Tranquilizer Combination of Butorphanol, Azaperone, and Medetomidine, and the Antagonists, Naltrexone, Atipamezole, and Tolazoline, in Black Bears (Ursus americanus) Post immobilization. Journal of wildlife diseases, 56(4), pp.933-936.

ABSTRACT: The tranquilizer combination of butorphanol, azaperone, and medetomidine (BAM) has shown good efficacy for immobilization of wildlife, including black bears (Ursus americanus). BAM is antagonized with a combination of naltrexone and atipamezole. We immobilized 19 adult captive wild caught black bears and, except for three bears that were euthanized immediately, bears were recovered with naltrexone and atipamezole. Tissue residues (0.01 ppm) for the tranquilizers butorphanol, azaperone, and medetomidine were detected in liver and muscle of all three bears euthanized on day 0 post injection (PI). Azaperone was not detected after 1 d PI. Residue for medetomidine was detected in two bears: in the liver 3 d PI and in the kidney 6 d PI. Butorphanol was reported in three bears: in fat 5 d PI, in kidney 6 d PI, and, surprisingly, in kidney, muscle, and fat 7 d PI. No tissue residues were detected in the three bears euthanized at 8 d PI. Tissue residues for the antagonists, naltrexone and atipamezole, were detected in bears euthanized 2 and 6 d PI, but not in tissues from animals euthanized at 7 or 8 d PI

APPENDIX B: Wildlife Health Research In Press, & In Review Manuscripts

In press FY 2020-21

Bian J, Kim S, Kane SJ, Crowell J, Sun JL, Burnett E, Christiansen J, Saijo E, Moreno JA, Pritzkow S, Soto C, Kreeger TJ, Balachandran A, Mitchell G, **Miller MW**, Nonno R, Vikøren T, Våge J, Madslien K, Tran L, Vuong TT, Benestad SL, Telling GC. 2021. Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein. *PLoS Pathogens*: *in press*.

Conner, M. M., **M. E. Wood**, A. Hubbs, J. Binfet, A. Holland, L. R. Meduna, A. Roug, J. P. Runge, T. D. Nordeen, M. J. Pybus, **M. W. Miller**. 2021. The relationship between harvest management and chronic wasting disease prevalence trends in western mule deer herds. *Journal of Wildlife Diseases* 57(4): *in press.*

Miller MW, & **Wolfe LL**. 2021. Inferring chronic wasting disease incidence from prevalence data. *Journal of Wildlife Diseases* 57(3): *in press*. (doi: https://doi.org/10.7589/JWD-D-20-00216)

Miller MW, & **Wolfe LL**. 2021. Prion disease in cervid species. In *Fowler's Zoo and Wild Animal Medicine Current Therapy*, 10th edition. R. E. Miller, P. P. Calle, N. Lamberski (eds.). W. B. Saunders, New York. *In press*.

Submitted for publication FY 2020-21 but still in review as of 30 June

Fisher MD, Prioreschi RA, **Wolfe LL**, Runge JP, Swanson HM, **Miller MW**. 2021. Lions and prions and deer reprise. *In review.*

Wolfe LL, **Fox KA**, **Griffin KA**, **Miller MW**. 2021. Mountain lions resist long-term dietary exposure to chronic wasting disease. *Revised, in review.*