COLORADO PARKS & WILDLIFE

Wildlife Research Reports

Wildlife Health

July 2021—June 2022

WILDLIFE RESEARCH REPORTS

JULY 2021-JUNE 2022

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 2021 through June 2022. These research efforts represent both short-term and long-term projects in various stages of completion. Projects are designed to provide tools and information to benefit conservation and management of wildlife in Colorado. In addition to research, the wildlife health program provides a number of services to aid CPW in managing Colorado's wildlife.

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

Wildlife health management services during 2021–2022 continued to emphasize detection of important wildlife health problems via examination of field case submissions and conducting disease diagnostics. The wildlife health program also provided training, field, and laboratory functions for many other CPW management and research programs.

In addition to these more general activities, we continued to support statewide CWD surveillance efforts by facilitating sample processing, overseeing database entry and quality control, and providing sampling training, focused testing services, and targeted surveillance.

Numerous collaborators supported our work. First and foremost, are the CPW field personnel across the state who report sick or dead wildlife and collect and transport carcasses and samples to support wildlife health diagnostics and disease surveillance. Additionally, members of the general public are invaluable in their prompt reporting of sick wildlife to local field offices. Such reports are the foundation of many wildlife health investigations. We also continued working in collaboration with the Colorado Department of Public Health & Environment and the Colorado Department of Agriculture to prevent, investigate, and (as needed) control 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, United States Department of Agriculture – Agricultural Research Services, World Wildlife Fund, Advantage Bio Consultants, Species Conservation Trust Fund, Wildlife Pharmaceuticals, Texas A&M Veterinary Diagnostic Laboratory, Wyoming State Veterinary Laboratory, and Wyoming Game and Fish Department.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

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

Period Covered: 1 July 2021–30 June 2022

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 ~430 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 1,700 acres at the Soapstone Natural Area and Meadow Springs Ranch black-footed ferret reintroduction site were treated with oral vaccine and/or insecticidal dust.

Previous Colorado Parks and Wildlife (CPW) research evaluating prairie dogs' serological response to a more economical vaccine dose (0.2x) has demonstrated positive

antibody responses (~26% seroconversion). However, the magnitude of this response is less than the seroconversion (>50%) observed in a study using captive prairie dogs. [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 2020, we distributed 8x baits during field trails at 50 baits/acre on black-tailed prairie dog plots. Antibody responses to the 8x dose baits in free-ranging prairie dogs averaged ~40% positive overall. No adverse effects of vaccination with 8x baits were observed. These results suggest that some individuals (adult females) may consume multiple baits, depriving younger prairie dogs of the opportunity to find and consume vaccine baits. Increasing bait density is likely to boost bait uptake in juvenile prairie dogs, which is needed to provide greater population-level protection from plague. [7, 8]

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

Methods

Study areas (6) included plots/colonies at Soapstone Prairie Natural Area/Meadow Springs Ranch, owned by the City of Fort Collins. Final selection of study colonies and plots followed field observations conducted in June 2021 to determine habitat quality and prairie dog population health. We included two triplicates (1x EDTA and 8x at 50 and 100 baits/acre) at sites SNA-11 and SNA-Round Butte (RB; Fig. 1).

In 2021, we captured and sampled free-ranging black-tailed prairie dogs on plots where 8x baits at 100 baits/acre and 1x EDTA baits at 50 and 100 baits/acre were administered (Fig. 1). This design generated serology data to compare antibody responses from prairie dogs captured on plots receiving 8x and 1x EDTA vaccine baits at both standard and increased bait density.

Antibody responses to 8x and 1x EDTA dose baits will also be compared with data collected during a previous dose response field study.

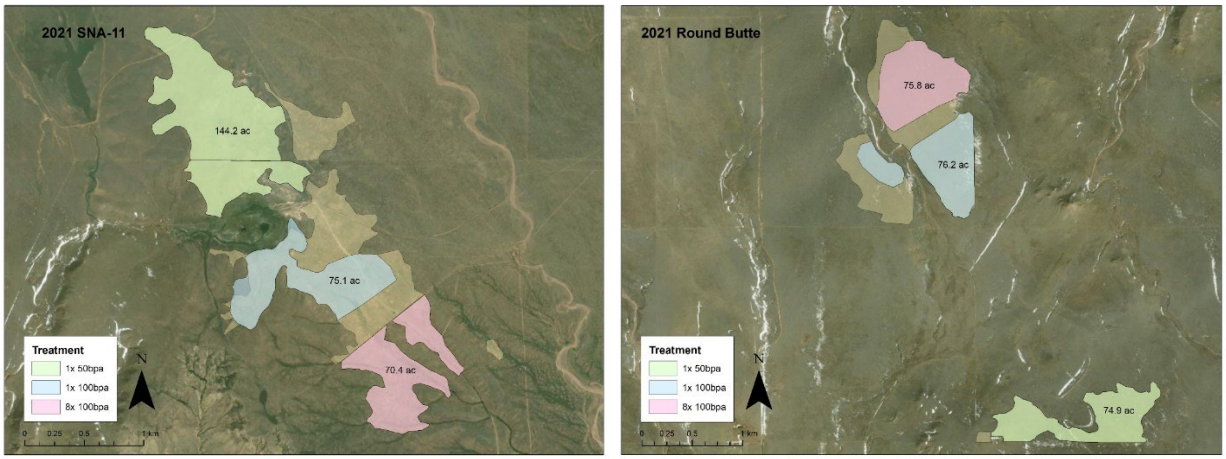

Figure 1. Treatment area at the Soapstone Natural Area Colony SNA-11(left panel) and Colony SNA-RB (right panel). 1x EDTA vaccine at 50 baits/acre (green), 1x EDTA vaccine at 100 baits/acre (blue) and 8x vaccine at 100 baits/acre (pink) treatment areas are shown.

Preliminary Results

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 2021 (Fig. 2). We will use these data to monitor responses to plague management though time.

We distributed ~273,500 doses of vaccine in ~171,200 baits on ~2,257 acres at the Soapstone Prairie and Meadow Springs Ranch complex. We also applied experimental vaccine baits (1x EDTA and 8x dose) on ~515 acres on 2 research plots (Fig. 1) and captured and sampled 196 prairie dogs on these plots in 2021. At SNA-11, 31-45% of the captured prairie dogs were seropositive for the V antigen (stimulated by the vaccine). On the SNA-RB plot, 34-50% were seropositive. We also assisted the City of Fort Collins Natural Areas program with spotlight surveys for black-footed ferrets in October 2021. Our CPW crew operated a spotlighting vehicle for 4 nights (~40 hours).

Future Efforts

We will continue to analyze data collected in 2020 and 2021 and prepare for continued field research in 2022. In 2022, we will continue vaccine bait manufacture, monitoring efforts and research to optimize the vaccine dose/bait, baits/acre and bait size 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.

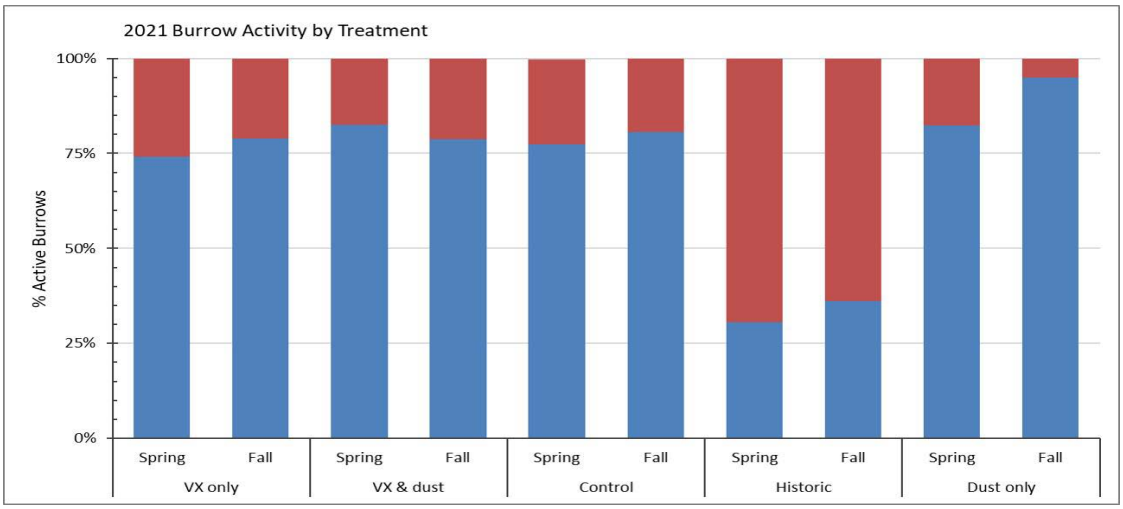

Figure 2. Proportion prairie dog burrows scored as active (blue) and inactive (red) during surveys conducted in the spring and autumn 2021 on colonies receiving plague management at the Soapstone Prairie and Meadow Springs Ranch complex.

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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 2021–30 June 2022

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 and other plague management tools. In 2013-15, as part of a multi-state research project, Colorado Parks and Wildlife distributed experimental vaccine and placebo baits on black-tailed prairie dog colonies on the two city of Fort Collins properties.[8] Continued collaboration with the city of Fort Collins to provide plague management support for the additional untreated ~1,500 – 2,000 acres while also continuing to evaluate plague management tools in select areas will help to ensure that stable populations of black-tailed prairie dogs exist to support black footed ferrets and the numerous other wildlife species they support.[1–7]

As an extension of long-term research, we have developed practical approaches for preventing plague outbreaks in prairie dog colonies, and we have managed plague on ~1,500 to 2,200 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, 10] 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 2016-21.

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

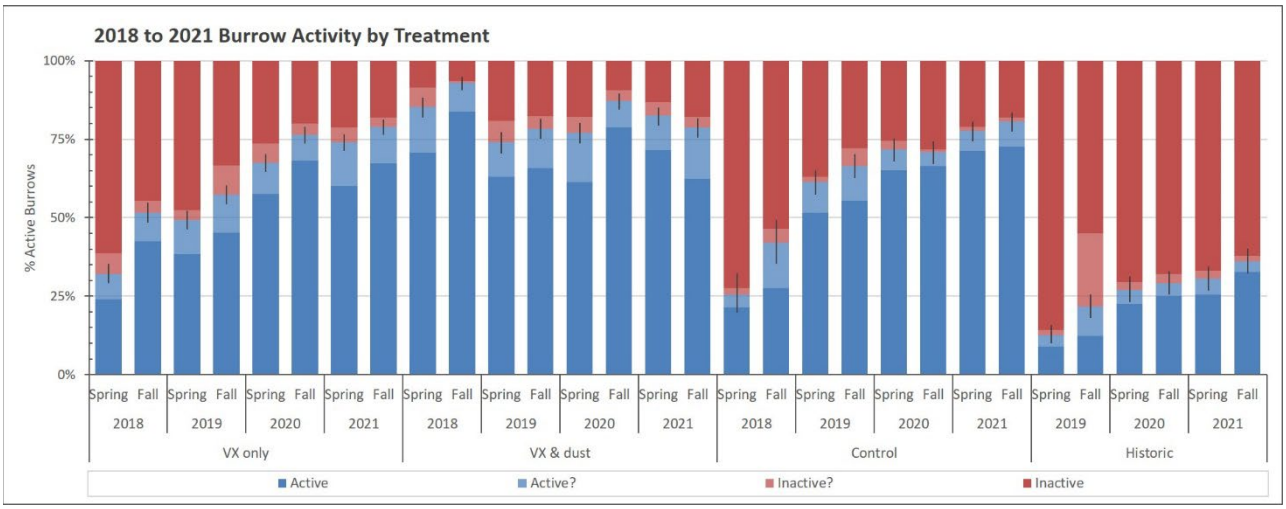

Figure 1. Proportion prairie dog burrows scored as active (blue), likely active (light blue), inactive (red) and likely inactive (light red) during surveys conducted in the spring and autumn 2018-2021 on colonies receiving plague management (vaccine, dust) or no treatment (control, historic) at the Soapstone Prairie and Meadow Springs Ranch complex.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Antibody responses of free-ranging black-tailed and Gunnison's prairie dogs to oral plague vaccine treated with (EDTA) at standard and high bait density

Period Covered: 1 July 2021–30 June 2022

Principal Investigators: Dan Tripp

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

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 (*Cynomys gunnisoni*).[3] 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 Gunnison's prairie dogs.

With the implementation of the NRCS Black-footed Ferret Initiative and the Programmatic Safe Harbor Agreement, Colorado has become an important location for the recovery of the black-footed ferret. Six release sites have been selected since 2013 with ~430 ferrets released. 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.[4]

Plague management in Colorado has evolved as new disease management tools have been developed.[5, 6] In 2021, about 2,200 acres at the Soapstone Natural Area and Meadow Springs Ranch black-footed ferret reintroduction site were treated with oral *Yersinia Pestis* Vaccine (YPV; Colorado Serum Company, Denver, CO). An additional ~2,200 acres of Gunnison's prairie dog habitat were treated with oral vaccine in 2021.

We recently conducted trials with captive prairie dogs to assess antibody responses to different doses of oral plague vaccine (CPW unpublished data). We evaluated 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.[7] A 3-day vaccine titration method used by USGS underestimated by ~8-fold the true amount of virus nominally designated as the standard "5×10^7 pfu" vaccine dose.

In 2020, we distributed ~4.8×108 TCID50 ("8x") baits during field trails at 50 baits/acre on black-tailed prairie dogs plots and 40 baits/acre on Gunnison's prairie dog plots. Antibody responses to the 8x dose baits in free-ranging prairie dogs averaged ~37% and 47% positive overall in black-tailed and Gunnison's prairie dogs respectively. No adverse effects of vaccination with 8x baits were observed. These results suggest that some individuals (adult females) may consume multiple baits, depriving younger prairie dogs of the opportunity to find and consume vaccine baits. Increasing bait density is likely to boost bait uptake in juvenile prairie dogs, which is needed to provide greater population-level protection from plague.6, 8

Recent in vitro work with YPV (K. 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 breaks up large aggregates of live vaccine virus, thereby making more individual virus particles biologically available. This improved bioavailability was demonstrated in vivo during a trial in which ~86% of captive prairie dogs that voluntarily consumed a bait containing a 1x dose of vaccine treated with 0.02% EDTA (hereafter 1x EDTA) had positive antibody responses to vaccination (CPW unpublished). No adverse effects of vaccination with 1x EDTA baits were observed. In light of these findings, further investigation of free-ranging prairie dogs' antibody responses to vaccine dosage (8x and 1x EDTA) and bait density is needed.

Methods

Gunnison's prairie dog study areas (6) included plots/colonies in South Park and the Gunnison Basin. We included two triplicates (1x EDTA and 8x at 40 and 80 baits/acre) at Cabin Creek SWA, BLM-18, Charlie Meyer SWA and Spinney Mountain. Black-tailed prairie dog study areas (6) included plots/colonies at Soapstone Prairie Natural Area/Meadow Springs Ranch, owned by the City of Fort Collins. We included two triplicates (1x EDTA and 8x at 50 and 100 baits/acre) at sites SNA-11 and SNA-Round Butte.

In 2021, we sampled free-ranging black-tailed prairie dogs on plots where 8x baits at 100 baits/acre and 1x EDTA baits at 50 and 100 baits/acre were administered. We also captured and sampled free-ranging Gunnison's prairie dogs on colonies receiving 8x dose baits at 80 baits/acre and 1x EDTA dose baits at 40 and 80 baits/acre. All vaccine baits were administered as part of on-going plague management programs. This design provided serology data to compare antibody responses from prairie dogs captured on plots receiving 8x and 1x EDTA vaccine baits at both standard and increased bait density. Antibody responses to 8x and 1x EDTA dose baits were also compared with data collected during a previous dose response field study.

Preliminary Results

We applied experimental vaccine baits (1x EDTA and 8x dose) on ~515 acres on two sites at the Soapstone Prairie and Meadow Springs Ranch complex and captured and sampled 196 prairie dogs on these plots in 2021. At SNA-11, 31-45% of the captured prairie dogs were seropositive for the V antigen (stimulated by the vaccine). On the SNA-RB plot, 34-50% were seropositive (Fig. 3).

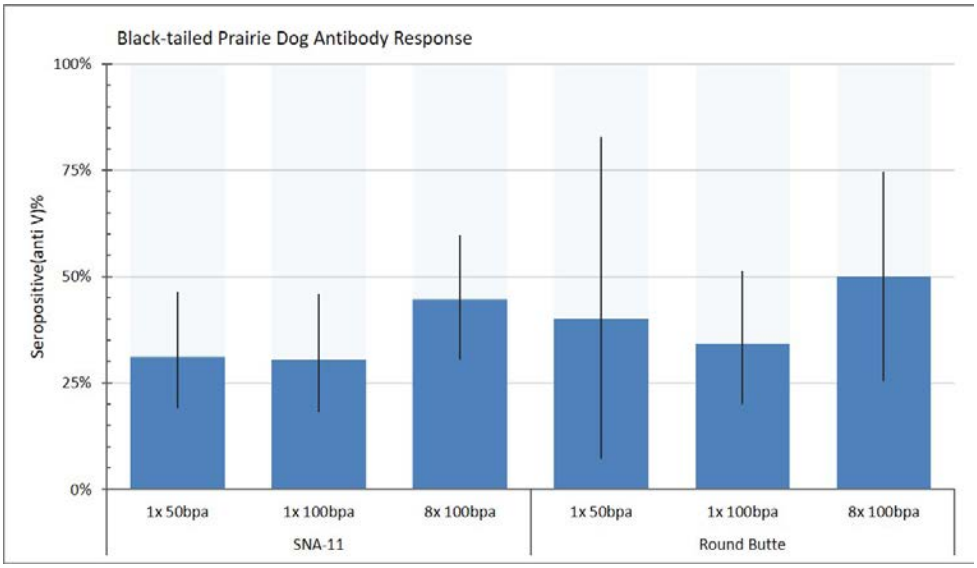

Figure 3. Proportion of vaccinated black-tailed prairie dogs with detected serum antibodies to *Yersinia pestis* V antigen ("seropositive") 28–43 days after receiving vaccine (RCN-F1-V307) orally at the 1x EDTA and 8x dose at 50 and 100 baits/acre.

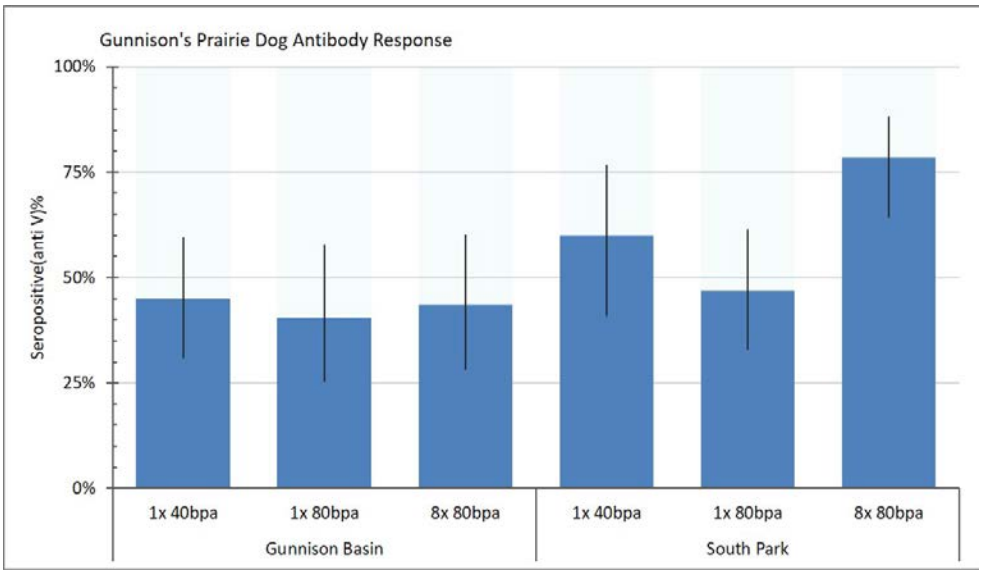

Figure 4. Proportion of vaccinated Gunnison's prairie dogs with detected serum antibodies to *Yersinia pestis* V antigen ("seropositive") 28–40 days after receiving vaccine (RCN-F1-V307) orally at the 1x EDTA and 8x dose at 40 and 80 baits/acre.

We applied experimental vaccine baits (1x EDTA and 8x dose) on ~280 acres on two Gunnison's prairie dog research sites and captured and sampled 255 prairie dogs on these sites in 2021. At Gunnison Basin sites, 41-45% of the captured prairie dogs were seropositive for the V antigen (stimulated by the vaccine). On the South Park sites, 47-78% were seropositive (Fig. 4).

Preliminary analysis of field serology data indicate that adult female Gunnison's prairie dogs are ~3.0 times more likely to seroconvert when 1x vaccine is treated with EDTA prior to bait manufacture. Furthermore, juvenile Gunnison's prairie dogs are ~3.4 times more likely to seroconvert when 1x EDTA treated baits are used. Adult male Gunnison's prairie dogs were ~2.0 times more likely to seroconvert when the bait density was increased from 40 to 80 baits/acre. The addition of EDTA to the bait manufacture protocol is a cost effective method to boost seroconversion in field vaccinated Gunnison's prairie dogs.

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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 2021–30 June 2022

Principal Investigators: Dan Tripp

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

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

Previous CPW collaboration with multiple partners has aided the development of vaccine bait distribution equipment for use on All-Terrain Vehicles (ATV).[9] Bait distribution with this equipment is 10-15 times more efficient than distribution on-foot. However, this equipment 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 also designed and built 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 increased 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 12 low-pressure, ATV mountable dusters (Fig. 1). These 12 units were field tested at Soapstone Prairie Natural Area in 2021. Fort Collins Natural Areas staff dusted over 65,840 burrows and the mean amount of dust dispensed was 6.37 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 therefore retrofitted 6 of the existing units with resettable breaker fuses to eliminate excessive fuse trips. New in 2021, we worked with a designer/fabricator to construct new uptake-vent tubes and dust vessel lids. These components will replace broken or worn first generation equipment. This collaboration also produced a new prototype “2.0” ATV duster that will serve as a testing platform for upgraded components and designs. In 2022, we will build and test additional components and prototype ATV dusters.

In 2021, we continued to perform needed maintenance and repairers on the existing bait distribution equipment and we were able to complete all scheduled bait distribution. We collaborated with a local software engineer to troubleshoot, repair and upgrade electrical and mechanical components in the existing units. We also collaborated with a local 3D design and print company to produce custom replacement parts. These repairs and upgrades will be tested in 2022. We continue to consult with engineers with the World Wildlife Fund to design and fabricate a new generation of more robust bait distribution equipment. However, this endeavor continues to be fraught with delays and missed deadlines.

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

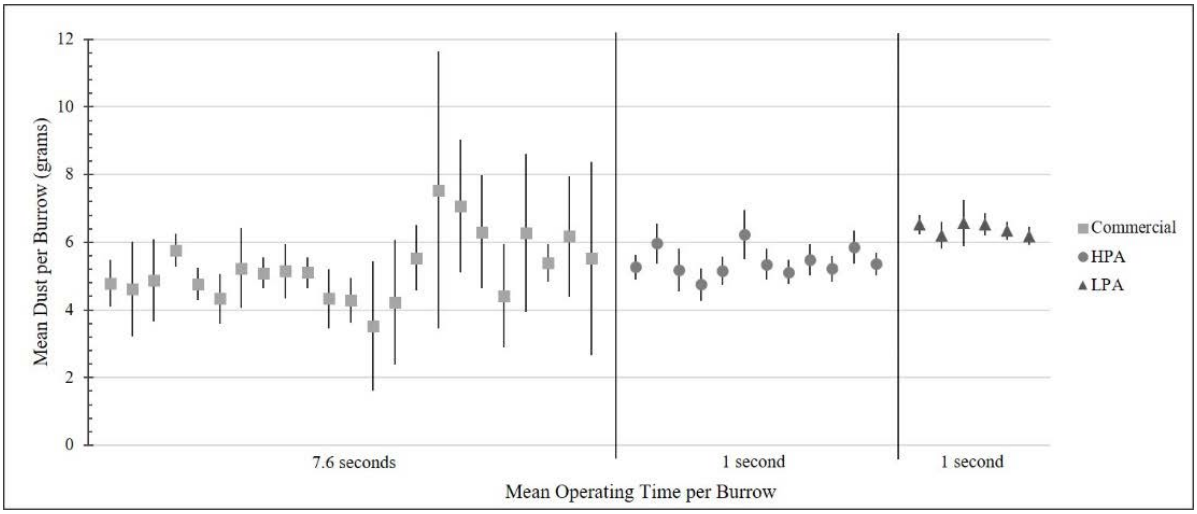

Figure 2. We field tested six low-pressure ATV dusters at Soapstone Prairie Natural Area in 2021 and compared performance to commercial and HPA dusters. The mean grams dust dispensed per burrow for each of the six units and time required at each burrow is shown. Error bars are 95% confidence intervals.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Clearance of *Mycoplasma ovipneumoniae* in captive bighorn sheep

Period Covered: 1 July 2021–30 June 2021

Principal Investigators: Mary E Wood, William H Edwards, Jessica E Jennings-Gaines, Mariah Gaston, Peach Van Wick, Sierra Amundson, Samantha E Allen, Lisa L. Wolfe

Respiratory disease presents a significant barrier for bighorn sheep (*Ovis canadensis*) conservation. Multiple bacterial pathogens contribute to the disease complex including *Mycoplasma ovipnuemoniae* and several Pasteurellaceae species (Besser et al. 2008; Post 1962; Miller et al. 2011). Additional pathogens may also contribute including sinus tumors, lungworms (*Protostrongylus spp.)*, psoroptes mites (*Psoropes ovis*), and respiratory viruses (Fox et al. 2011; Forrester et al. 1971; Honess and Frost 1942; Howe et al. 1966). The convergence of multiple pathogens and ecological influences at the landscape level create a complex system for management that may necessitate stepwise or combined management approaches. *Mycoplasma ovipneumoniae* can impair mucociliary clearance of bacterial pathogens and may predispose an animal to infection with other bacteria (Dassanayake et al. 2010). Clearance of virulent strains *M. ovipneumoniae* is an important step to improve population health and allow for clearance of other respiratory pathogens.

Proposed therapies for *Mycoplasma* infections include fluroquinolone, tetracycline, macrolide, and aminoglycoside antibiotics (Gopalakrishnan et al. 2019). Several of these were administered to domestic and wild sheep (Sirochman et al. 2012, Weiser et al. 2009, Politis et al. 2019, Gopalakrishnan et al. 2019) but, to date, there are no published reports of successful treatment of *M. ovipneumoniae* in bighorn sheep.

We opportunistically treated *Mycoplasma ovipneumoniae* infection in six bighorn lambs and five bighorn yearlings at two captive research facilities. Initial treatment approaches were on a single bighorn lamb with a largely trial and error approach. We tried a variety of antibiotic and supportive therapies to clear *M. ovipneumoniae* without success. Clinical signs persisted despite treatment, and *M. ovipneumoniae* was still present after two months. Thereafter we switched to twice daily oral doxycycline and treated for eight weeks for successful clearance. This therapy was repeated with 10 additional bighorn sheep. Lambs were treated with doxycycline mixed in milk replacer, while yearlings were treated with doxycycline powder mixed in moistened, pelleted feed. All animals remain clear of *Mycoplasma ovipneumoniae* two or more years later.

We compiled all treatment data from work conducted at both captive research facilities and prepared a manuscript describing this successful clearance of *Mycoplasma ovipneumoniae* during this fiscal year. Prolonged oral antibiotic therapy is difficult to achieve in a free-ranging setting, but there may be select opportunities for treatment in high priority conservation efforts where temporary holding and treatment of bighorn sheep may be desirable. This work may help facilitate conservation efforts for high priority bighorn populations west wide.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Development of naturally degrading wildlife collar drop-off mechanisms

Period Covered: 1 July 2021–30 June 2022

Principal Investigators: Mary E. Wood, Mark Fisher, Mat Alldredge, Pauline Nol, Eli Burns, Caleb Hollingsworth, Adam Lujan

Wild ungulates are captured and collared to support a wide array of research and management operations in Colorado. The battery life of these collars is often far less than the potential lifespan of the animal. To minimize the length of time that an animal is wearing a collar, researchers utilize various drop-off mechanisms to ensure that a collar comes off an animal once data collection is complete. While automated remote collar drop-off mechanisms are currently available from manufacturers; they often come with a substantial cost and may be prone to failure. Naturally degradable materials such as cotton spacers have been utilized; however, timing of drop-off is highly inconsistent and these materials often last many years beyond the battery life of the collar. We worked with three CSU engineering and materials science students to investigate new materials and passive mechanisms to allow collars to fall off naturally over a predicted timespan. This would reduce costs for collars by eliminating the need for remote collar drop off mechanisms in many studies and also reduce the length of time that many animals may need to wear a collar.

We sponsored three senior engineering students to help design a naturally degrading collar drop-off device for their senior design project. The students researched both mechanical and automated drop-off options and ultimately settled on a simple design utilizing the degradable polymer PolyCaproLactone (PCL). This polymer is used in many medical applications for its ability to degrade in a predictable manner and can be tailored to fail within a specific period. PCL is a 3D printable material, allowing for rapid prototyping, easy manufacturing, and the ability to manufacture the device in-house if necessary. The physical design of the drop-off was modeled after the currently used cotton spacer. This allows field personnel to easily transition to the new device and does not compromise collar shape or function. Since PCL degrades at a steady rate based on ambient conditions, altering the thickness of the device allows you to customize the time that the device will remain intact. This allows field personnel to customize the length of time that a collar will stay on an animal based on the predicted battery time of the collar.

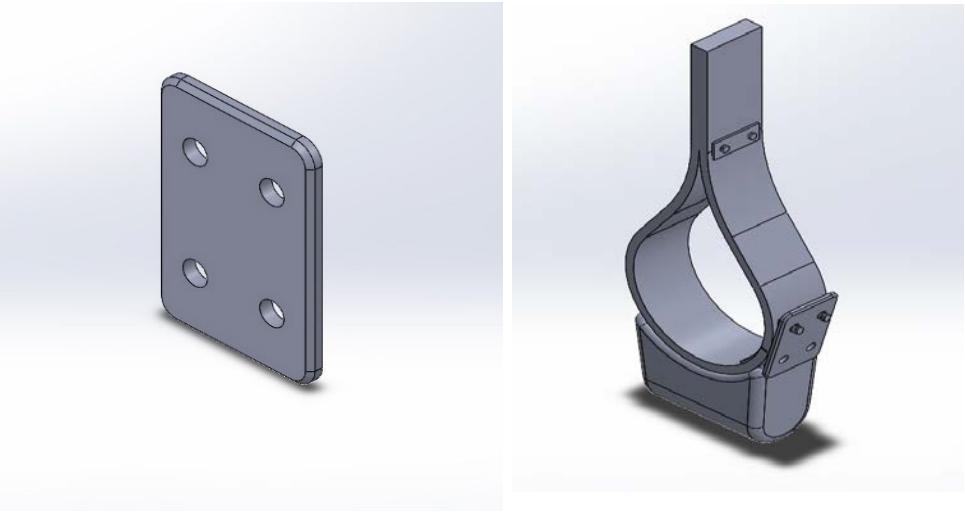

Figure 1: Final Device Design (left), and full collar assembly with device (right)

Preliminary evaluation involved tensile testing of devices with an array of different thicknesses for baseline tensile strength. Devices were placed into environmental chambers to simulate ambient conditions in Colorado. After various periods of time in the environmental chambers, devices were removed for tensile testing. Preliminary laboratory data supported a steady rate of degradation of the device.

Further research is underway to test the devices at various thicknesses on static collars under natural environmental conditions. A subset of devices will be attached to collars and fitted on captive animals for long-term assessment under natural conditions.

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 2021 – 30 June 2022

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 evaluated whether tolazoline and vatinoxan could improve the cardiovascular and respiratory side effects of the drug combination butorphanol-azaperone-medetomidine (BAM). We hypothesized that elk receiving tolazoline and vatinoxan at 20 minutes post induction could have a higher heart rate, respiratory rate, oxygen saturation (SpO2), and arterial partial pressure of oxygen (PaO2), and lower mean arterial blood pressure (MAP) than elk not receiving these drugs. Immobilization with BAM at a dose of 0.15 mg/kg butorphanol, 0.05 mg/kg azaperone, and 0.06 mg/kg medetomidine caused hypoxemia, bradycardia, and moderate hypertension. Because of the severe hypoxemia observed, all animals received intra-tracheal oxygen throughout the immobilization. Intramuscular (IM) tolazoline at 2 mg/kg had no significant effect on heart rate or respiratory rate as compared to before administration. Intravenous vatinoxan at 3 mg vatinoxan per mg medetomidine caused a significant drop in blood pressure and increase in heart rate but no change in respiration nor did we measure any changes in oxygenation (Figure 1). The depth of sedation was unchanged after administration of either of the drugs. Further work is warranted to explore these drugs at different dosages and routes of administration.

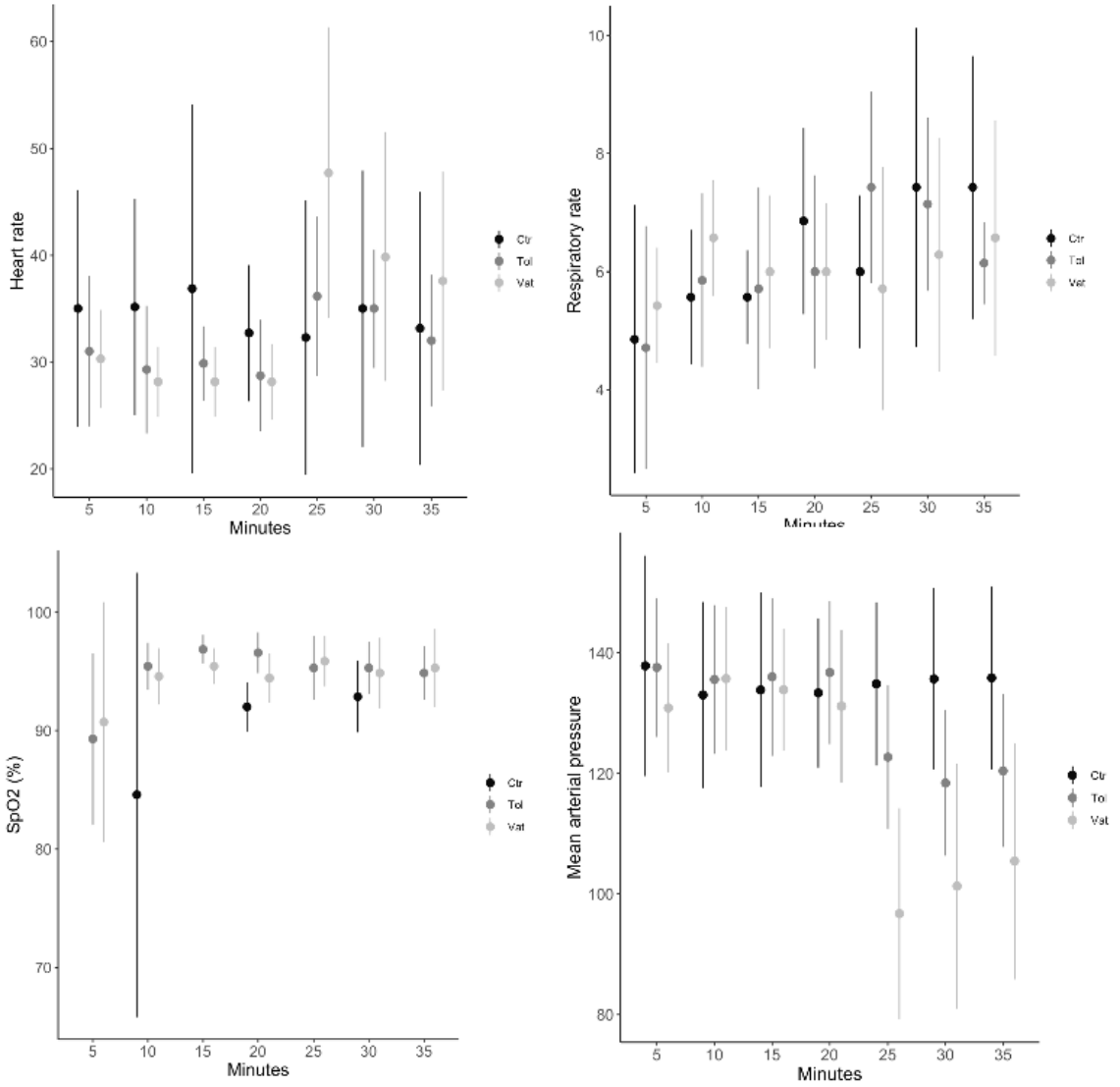

Figure 1: Heart rate, respiratory rate, sPO2, and mean arterial pressure in Rocky mountain elk immobilized with butorphanol-azaperone-medetomidine. Saline, tolazoline, or vatinoxan was administered at 20 minutes.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Assessment of a genetically-modified *Mannheimia haemolytica* and *Mycoplasma ovipneumoniae* vaccine in bighorn sheep.

Period Covered: 1 July 2021–30 June 2021

Principal Investigators: Mary E. Wood, Rohana Dassanyake, Robert Briggs, Fred Tatum, Michael Miller

Successful bighorn sheep (*Ovis canadensis*) management in Colorado and elsewhere appears dependent on preventing or limiting the effects of pneumonia epidemics in otherwise thriving herds (George et al. 2009). Periodic respiratory disease outbreaks cause extensive "all-age" mortality and/or sustained recruitment depression in bighorn populations throughout North America. Although a variety of agents have been identified in these outbreaks, leukotoxigenic *Pasteurellaceae* (especially *Mannheimia haemolytica* and *Bibersteinia trehalosi*) and *Mycoplasma ovipneumoniae* appear most consistently associated with bronchopneumonia in contemporary bighorn herds in Colorado (Wolfe et al. 2010, Miller et al. 2014, Sirochman et al. 2012, Wood et al. 2016).

An effective vaccine against important respiratory pathogens could improve bighorn management by protecting bighorn herds from catastrophic die-offs and/or ameliorating lamb losses caused by pneumonia. To date, the efficacies of vaccines in protecting bighorn sheep from pneumonia have varied widely. Some failed to prevent disease (e.g., Foreyt 1992), and others actually caused disease (Onderka et al. 1988; Miller et al. 2000).

Despite some promising experimental and field results, incorporating vaccines into bighorn management programs remains problematic. Conventional hand-injection methods are undesirable because they require handling of individual animals, and thus can be stressful to wild sheep and labor-intensive (Sirochman et al. 2012). Delivering vaccine via projectile syringe suffers from the similar limitations. Bighorn herds have effectively been treated orally with anthelmintics and antibiotics for many years (Feuerstein et al. 1980, Sirochman et al. 2012), and oral administration of vaccines to ruminants has been shown potentially efficacious (Bowersock et al. 1994a, 1994b); however, field application would require congregating bighorns at feeding stations. In order to achieve sufficient "herd immunity" and population-level protection from respiratory disease a substantial proportion of the populations likely will need to be vaccinated.

We hypothesized that a *M. haemolytica* or *B. trehalosi* strain containing a modified gene coding for antigenic but noncytotoxic leukotoxin (leukotoxoid) could be introduced into a wild bighorn population via intranasal vaccination (Miller et al. 2000, Miller 2001). This nonpathogenic, self-replicating organism theoretically could be passed from one animal to another through normal contact. It follows that vaccinating a small percentage of the population with a replicating, transmissible vaccine could rapidly lead to protection of a greater number of animals, thereby conferring population-level immunity.

Novel, genetically-modified live vaccine strains displaying many of the foregoing features have been developed and evaluated in cattle, domestic sheep and goats (Briggs et al. 1998, Tatum et al. 1998, Briggs and Tatum 1999, Briggs et al. 2012, R. E. Briggs unpubl. Data). Additional work by the US Department of Agriculture's Agriculture Research Service (Ames, Iowa) developed *Pasteurellaceae* vaccine carrier strains that are less likely to cause disease in bighorn sheep. In addition to improved attenuation, the new vaccine strain expresses chimeric antigens derived from *M. haemolytica* (leukotoxoid) and *M. ovipneumoniae* (heat shock protein 70 [HSP70] and elongation factor thermo unstable ([EF-Tu]; Jiang et al. 2016) to stimulate a broader immune response to key respiratory pathogens. A similar vaccine construct has proven effective in domestic cattle.

To assess safety, colonization, immunogenicity, and transmissibility of candidate vaccine strain we used five previously unvaccinated captive bighorns as primary vaccinates; each bighorn initially served as its own control. We administered vaccine in a powder form and mixed with moistened pelleted feed. Bighorn sheep were held in individual pens and given two hours to consume the vaccine. Safety was evaluated by observing for signs of morbidity or mortality over a 30-day period. Samples collected within 4 weeks prior to vaccination and on approximately day 7, 21, 35, and 90 post-vaccination were used to establish baseline and measure antibody responses and strain-specific colonization rates.

Four of the five sheep consumed a full dose of vaccine. One sheep consumed only a partial dose. All sheep were free from clinical signs of disease until day nine. One ewe developed clinical signs with respiratory disease and was treated with antibiotics and anti-inflammatories. The vaccine strain was recovered from a nasal swab collected on the day of clinical onset and serology from day 21 revealed a mild antibody response to the vaccine.

None of the remaining animals developed clinical signs of disease. Vaccine strain was not recovered from any of the remaining animals and none of the other animals developed serological responses to the vaccine. It appears that this vaccine construct is unlikely to provide sufficient immune response in bighorn sheep. Further work with other vaccine constructs focused on *Bibersteinia trehalosi* as a carrier may prove more efficacious at eliciting immune responses in bighorn sheep without causing clinical disease.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Multiplex PCR for multilocus sequence typing, Illumina short-read sequencing, and custom bioinformatics for bighorn sheep respiratory disease diagnostics

Period Covered: 1 July 2021–30 June 2022

Principal Investigators: Karen A. Fox, Kevin Blecha

For at least a century, bighorn sheep (*Ovis canadensis*) populations in North America have experienced declines due to mortality from respiratory diseases. Some bighorn populations have experienced dramatic and abrupt declines from outbreaks of acute pneumonia in all age classes, whereas other populations have experienced steady declines due to chronic respiratory disease in the herd and annual lamb mortality. Like respiratory diseases in domestic sheep and cattle, bighorn sheep respiratory disease is a polymicrobial and multifactorial problem that can be difficult to manage, especially in free-ranging populations. In recent years, much of bighorn sheep respiratory disease research has focused on a single pathogen, *Mycoplasma ovipneumoniae*. In both domestic (Alley et al. 1999) and bighorn (Besser et al. 2008) sheep, *M. ovipneumoniae* demonstrates low pathogenicity in the absence of coinfections. However, in combination with other bacterial infections, *M. ovipneumoniae* can predispose to pneumonia by causing ciliostasis that disables normal clearance of pathogens from the lungs (Dassanayake et al. 2010). Additionally, proliferation of the tissues in the upper respiratory sinuses (bighorn sheep sinus tumors) also predispose to lower respiratory infections, presumably through alteration of normal upper respiratory clearance mechanisms (Fox et al. 2015). As a result, fatal bacterial pneumonia infections in bighorn sheep typically include *Mycoplasma ovipneumoniae*, and pathogens from the Pasteurellaceae family including *Mannheimia haemolytica, Bibersteinia trehalosi, and Pasteurella multocida*.

Multilocus sequence typing (MLST) characterizes bacteria based on genetic variability in constitutive (housekeeping) genes, and allows comparisons of bacteria beyond the species designation. This approach has been used to trace outbreaks of diseases, and an MLST approach has been used to examine *Mycoplasma ovipneumoniae* (Cassirer et al. 2017) in bighorn sheep. However, bighorn sheep respiratory disease is a polymicrobial concern, and focus on a single pathogen limits diagnostic and management strategies. To create a broader approach to bighorn

sheep respiratory diagnostics, we created a single, culture-independent assay to detect and characterize *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mycoplasma ovipneumoniae*. The assay detects up to eight MLST loci for strain typing of each species, the Pasteurellaceae leukotoxin A gene (lkt A), and 16S rDNA sequences. The assay is based on a three-step approach: 1)Multiplex PCR to probe samples for targets (Figure 1) 2)Next generation sequencing to determine the genetic sequences of each target, and 3)Bioinformatics in the form of automated software to analyze genetic sequences (Figure 2). Results from MLST analyses can be displayed as phylogenetic trees (Figure 3), while results from 16S rDNA analysis are displayed as interactive pie graphs (Figure 4). This assay was originally designed to assess possible transfer of pathogens from domestic to bighorn sheep in the event of a bighorn sheep mortality from respiratory disease. However, the assay could be useful for many applications in bighorn sheep respiratory disease research and management.

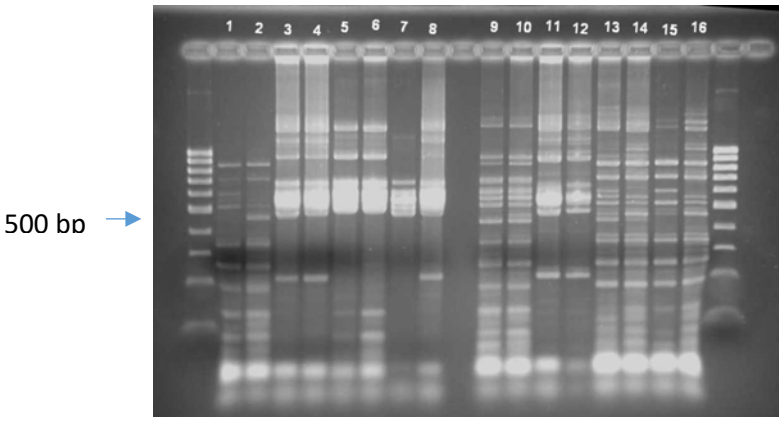

Figure 1. PCR gel showing amplification of (up to) 31 PCR products for bighorn sheep bacterial pathogen multiplex. Note very bright bands near 500 base pair (bp) marker. This indicates samples for which the PCR reaction has amplified abundant products of the desired length.

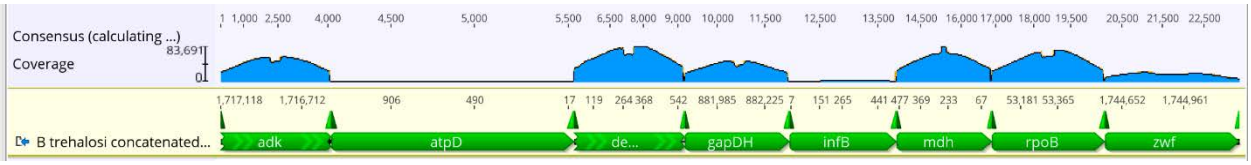

Figure 2. One example of the map to reference function used to retrieve MLST strain typing data as part of custom automated bioinformatics. *Bibersteinia trehalosi* concatenated reference shown with eight targeted genes represented by green bars at bottom of image, and primers represented by green triangles. Blue shading indicates depth of coverage – up to 83,000 reads.

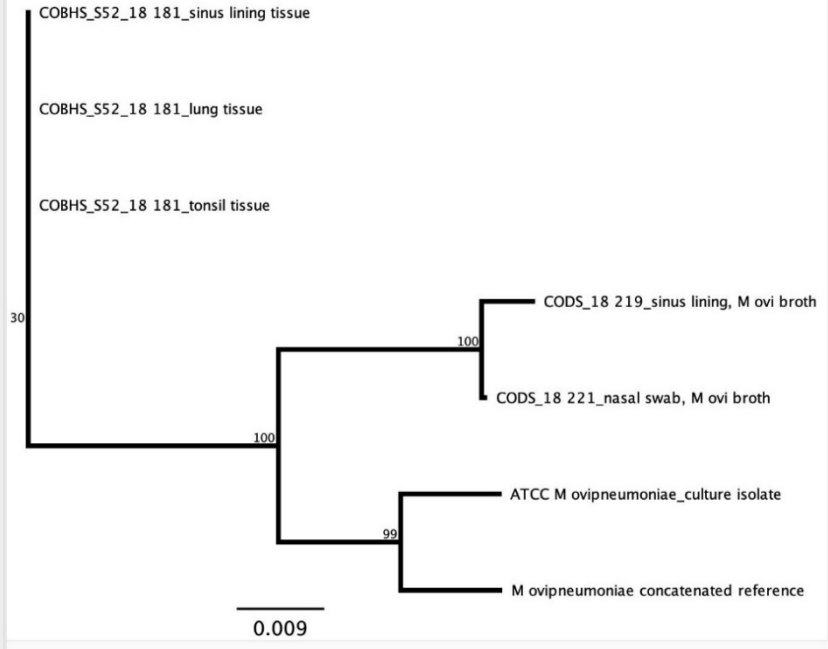

Figure 3. Example of a phylogenetic tree created from NGS strain typing of *Mycoplasma ovipneumoniae*. The tree is based on sequences retrieved from sequencing of multiplex PCR reactions followed by mapping MLST loci and concatenating gene sequences. *M. ovipneumoniae* was detected in bighorn (BHS) and domestic (DS) sheep samples, but there was no evidence of shared strains between bighorns and domestics.

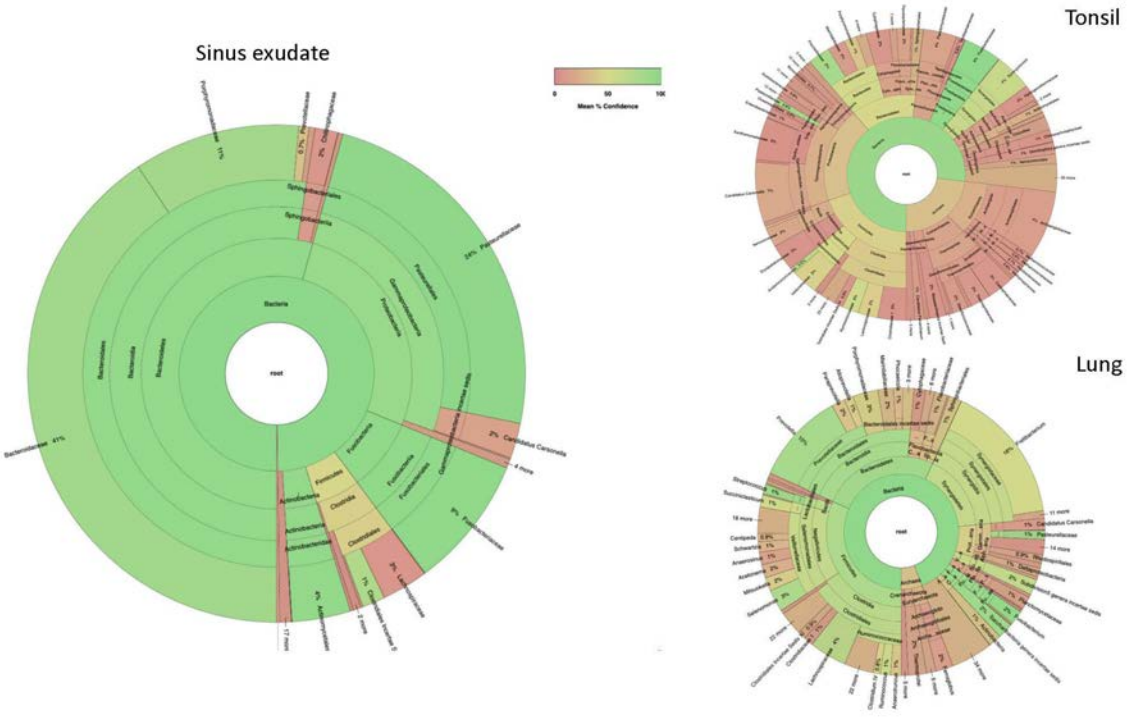

Figure 4. 16S gene analysis of samples from an apparently healthy domestic sheep with upper respiratory disease detected post-mortem. Less bacterial diversity is present in the sample of sinus ling tissue than in the lung or tonsil tissues. This suggests chronic upper respiratory disease, and helps to understand the shift in the bacterial population that can occur with chronic upper respiratory disease.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Optimizing chronic wasting disease diagnostics in elk

Period Covered: 1 July 2021–30 June 2021

Principal Investigators: Mary E. Wood, Hank Edwards, Terry Spraker, Karen Griffin

Chronic wasting disease (CWD) is a fatal, infectious disease caused by an abnormal protein called a prion (PrP^d). This disease affects members of the cervid family, and is now endemic across much of Colorado. To date, CWD has been detected in 40 of 54 deer herds, 17 of 42 elk herds, and 2 of 9 moose herds in Colorado.

There are currently three United States Department of Agriculture (USDA) approved tests available for CWD diagnosis in cervids through the United States National Animal Health Laboratory Network (NAHLN). The first test is immunohistochemistry (IHC) where tissues are fixed in formalin, embedded in paraffin, sectioned, and then stained with an antibody that binds to the CWD prion (O'rourke et al. 2000, Spraker et al. 2002). Stained sections are examined microscopically for evidence of PrP^d accumulation. This is considered the gold standard diagnostic test for CWD in the US; however, it is limited to evaluate only very thin cross-sections of tissue making it challenging to evaluate an entire piece of tissue. The other two tests are enzyme-linked immunosorbent assays (ELISA) where multiple pieces of a tissue are homogenated and then incubated with color-bound antibody that binds to the CWD prion. Then the optical density is measured to determine presence of PrP^d (Hibler et al. 2003, De Bosschere et al. 2006). These tests benefit from utilizing larger portions of tissue (200-300 mg) collected from multiple locations within the tissue. Two manufacturers have USDA approved ELISAs for CWD testing: Bio-Rad Laboratories (Hercules, CA), and IDEXX Laboratories (Westbrook, MA). The Bio-Rad ELISA has an added protein digestion step that is not used in the IDEXX. While the ELISA is currently used for the majority of hunter-harvest CWD screening, there is only one published paper directly assessing performance of the Bio-Rad ELISA as compared to IHC (Hibler et al. 2003) and there are no publications directly assessing performance of the IDEXX ELISA or estimating agreement between the two ELISAs. There are also no publications assessing performance of either ELISA on tonsillar tissue.

Challenges with chronic wasting disease diagnostics include limitations with the gold standard test, variations in tissue distribution of the disease, and possible variations in prion distribution among species. Early in the disease course, deposition of CWD prions is sparse and may be limited to certain tissues or even portions of a tissue, resulting in lower diagnostic sensitivity (Sigurdson et al. 1999, Spraker et al. 1997, 2004). Investigations into the pathogenesis and distribution of CWD in mule deer using IHC demonstrated earliest disease detection in retropharyngeal lymph nodes, followed by tonsils and lymphoid tissues along the intestinal tract, with later detection in the brain and spinal cord. (Sigurdson et al. 1999, Fox et al. 2006). Similar published work to assess early disease pathogenesis and distribution in elk is not available. In 2003, the Bio-Rad ELISA became available for rapid CWD diagnostics in the US. On initial validation of the test, 22% of CWD positive mule deer and 7% of CWD positive elk tested positive only in the retropharyngeal lymph node and not in the obex region of the brainstem, while all samples testing positive in the obex also tested positive in the retropharyngeal lymph node (Hibler et al. 2003). However, only 15 sets of positive elk tissues were available for evaluation. Paired white-tailed deer samples tested with both ELISA and IHC were CWD positive in the retropharyngeal lymph node and not in the obex in 20% of animals tested, while all samples testing positive in the obex also tested positive in the retropharyngeal lymph node (Keane et al. 2008).

On further evaluation, researchers identified slight differences in CWD detection between elk and deer. Spraker et al. (2004) noted that out of 226 CWD positive captive elk evaluated using IHC, 12% were positive only in the obex, 14% were positive only in the retropharyngeal lymph node, and 0.8% were positive only in the tonsil. Haley et al. (2016) reported similar results in captive elk tissues. There have been comparatively few assessments of tonsils as a post-mortem diagnostic sample for CWD. Tonsils are an easy tissue to collect and can provide a useful alternative to retropharyngeal lymph nodes or obex in the event that other tissues are unavailable (Miller & Williams 2002). However, Spraker et al. (2004) noted that only approximately 65% of CWD cases in captive elk were positive via IHC on tonsil while 80% were positive in the obex and 84% were positive in the retropharyngeal lymph node. This may suggest that tonsil, while a preferred tissue in deer, may not be preferred in elk.

Research evaluating approved diagnostic tests for CWD among different cervid species and tissues is limited, particularly in elk, and there are differences in disease pathogenesis between deer and elk. High-volume field sampling for surveillance and monitoring purposes in free-ranging populations is costly and time-consuming. Colorado Parks and Wildlife currently utilizes retropharyngeal lymph nodes as the preferred sample for CWD testing in all cervids, including elk, using the Bio-Rad ELISA. Adding additional tissue sampling and diagnostic fees to test paired samples on all elk is not cost-effective for our current surveillance and monitoring program. Assessing approved CWD diagnostic test performance on a variety of tissues will inform us on preferred tissues and diagnostic tests for CWD surveillance programs, accuracy of CWD prevalence estimates in elk, and whether a correction should be made to account for diagnostic sensitivity. This work will also help inform collaborative efforts across jurisdictional lines to understand disease transmission dynamics on the landscape. Test and tissue selection

may be important to consider when attempting to compare data among jurisdictions that utilize different tests or tissues.

Retropharyngeal lymph nodes, tonsils, and obex were opportunistically collected from hunter-harvested elk as well as elk submitted for necropsy in both Colorado and Wyoming in the fall of 2021. A single retropharyngeal lymph node was initially screened by ELISA (either Bio-Rad or IDEXX) for hunter results. To date, we have collected 719 sets of elk tissues with 33 of those from positive elk on preliminary screening. Additional sample collection is planned for the 2022 hunting seasons and diagnostic testing will be conducted after the 2022 sample collection. After initial screening, 50 sets of positive tissues and a subsample of 250 tissue sets from those testing negative on preliminary screening will be used in the study. Tissues will be tested using IHC and the two ELISAs with results compared among tissues and tests. All animals included in the study will be genotyped for the prion protein gene alleles at codon 132 as previously described (O'Rourke et al. 1999).

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

Sinusitis and conjunctivitis in a wild turkey (*Meleagris Gallopavo*) associated with *Avibacterium*-like bacteria

Period Covered: 1 July 2021–30 June 2022

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

In January of 2020, a Merriam's wild turkey (*Meleagris gallopavo merriami*) was observed with periocular swelling and periocular skin crusting in Pueblo County, Colorado. The wild turkey was diagnosed with severe catarrhal and fibrinous sinusitis and conjunctivitis (Figures 1&2). Wild turkey populations are generally stable in Colorado, with no evidence of population limiting diseases. However, historic concerns for population declines have included disease effects from *Mycoplasma* spp. bacteria (Adrian 1984). Mycoplasmosis can cause upper respiratory diseases such as conjunctivitis and sinusitis that give birds an appearance of periocular swelling (Davidson et al. 1982), and may cause reproductive losses (Rocke et al. 1988). Other diseases that may cause periocular swelling or crusting in wild turkeys include avian pox, bacterial dermatitis, and lymphoproliferative disease (Elsmo et al. 2016). One cause of periocular swelling in domestic chickens (*Gallus gallus domesticus*) is infectious coryza, caused by the bacteria *Avibacterium paragallinarum* (Anjaneya et al. 2013; Blackall and Soriano-Vargas 2020). Although this disease has not been reported in wild turkeys, it is commonly encountered in backyard poultry (Byarugaba et al. 2007; Clothier et al. 2019). The increasing popularity of backyard flocks in Colorado raises concerns for pathogen transmission between wild and domestic birds. Given concerns for wild and domestic avian health, we investigated the cause of disease in this wild turkey flock.

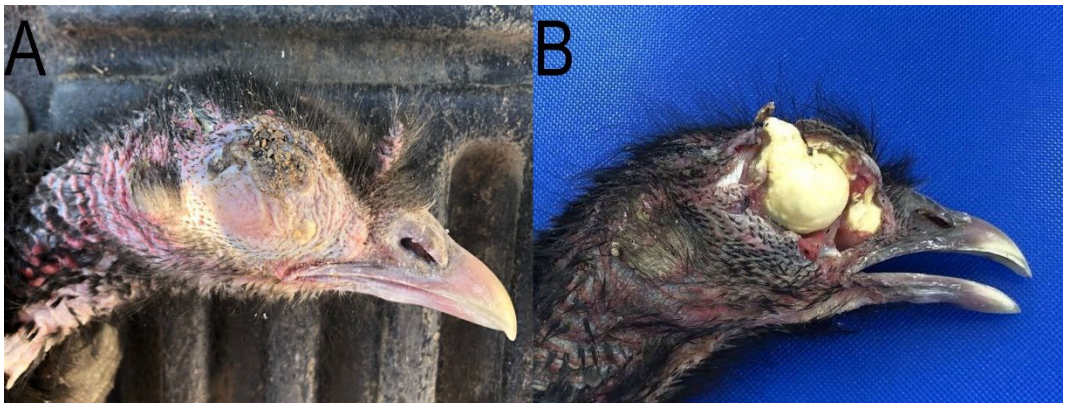

Figure 1. Head from the affected wild turkey. (A) Both eyes are swollen shut, with ulceration and crusting of the palpebral skin. (B) Removal of the palpebral skin reveals an extensive mat of dense fibrinous exudate, covering the right eye and communicating with the infraorbital sinus.

A novel clade of *Avibacterium* was detected in the exudates from the affected bird. While eight additional turkeys culled from the affected flock did not have clinical signs or gross lesions, histologically all had mild to moderate chronic sinusitis (Fig. 2D), and infraorbital cultures yielded the same novel clade of *Avibacterium* that was found in the symptomatic turkey (Figures 3&4). Negative culture results from a distant flock of wild turkeys, acquired with similar methods to the affected flock, suggested that this novel species of *Avibacterium* was not widespread throughout wild turkeys in Colorado.

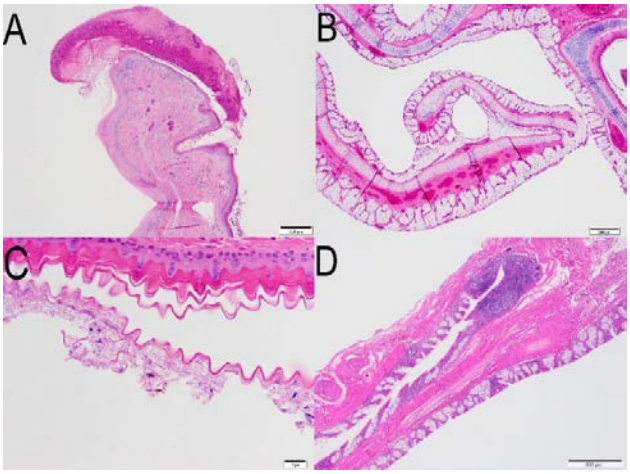

Figure 2. Histology from the symptomatic wild turkey (A-C) and an asymptomatic turkey in the flock (D). (A) The conjunctival epithelium is attenuated and covered by an extensive crust. The subepithelial stroma is expanded by congestion, edema, and lymphoplasmacytic inflammation. (B) The nasal scrolls are irregular in shape and collapsed. Note marked goblet cell hyperplasia. (C) The mucosal epithelium shows parakeratosis of the stratified squamous epithelium. Bacteria are present at the surface of, and embedded within, the hyperplastic keratin layer. (D) The submucosa is moderately expanded by lymphocytes and plasma cells, with mild epithelial goblet cell hyperplasia.

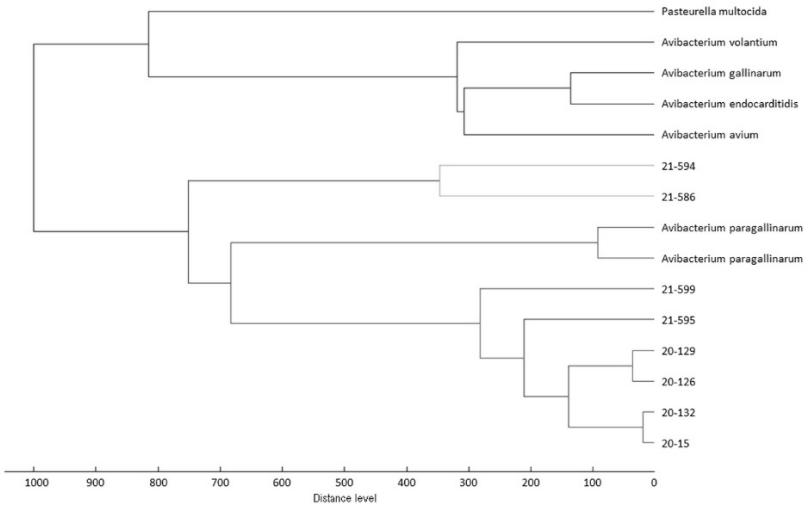

Figure 3. MALDI-TOF dendrogram of Pasteurellaceae isolates. Isolates from a symptomatic wild turkey (20-15), other wild turkeys from the affected flock (20-126, 20-129, and 20-132) and wild turkeys from a nearby, connected flock (21-595 and 21-599) cluster as a clade independent from *Avibacterium* references.

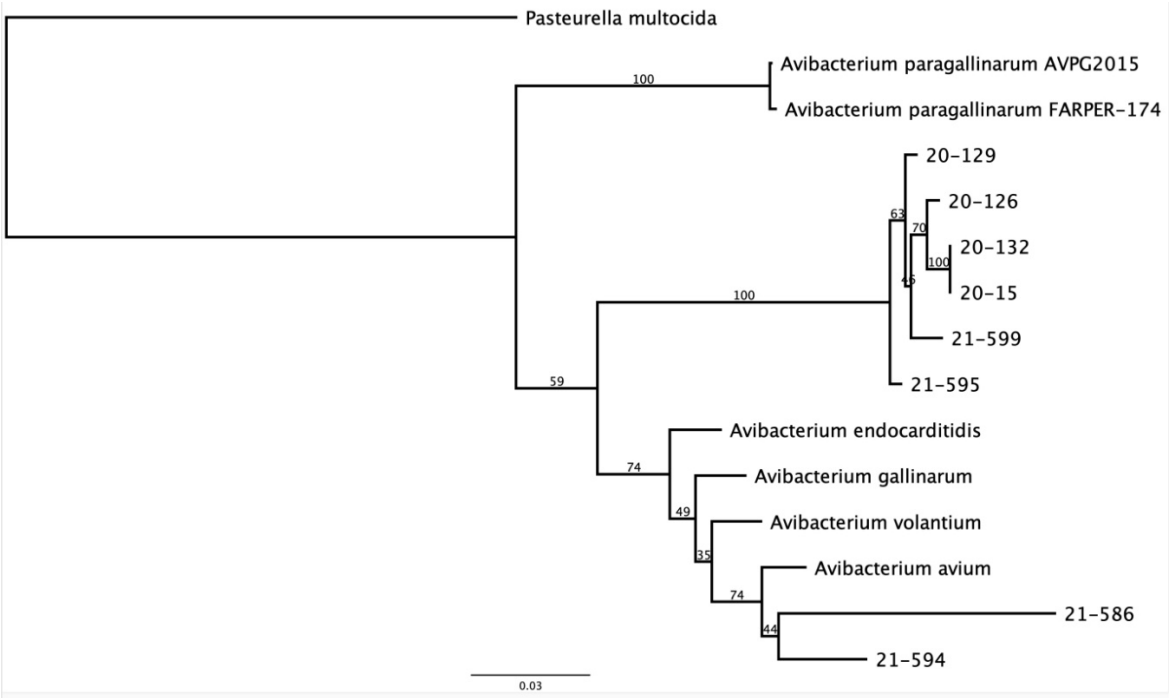

Figure 4. Maximum-likelihood phylogeny of Pasteurellaceae isolates collected from avian samples in Colorado, USA 2020-2021, including *Avibacterium* genus reference strains and outgroup Pasteurella multocida. Isolates from a symptomatic wild turkey (20-15), other wild turkeys from the affected flock (20-126, 20-129, and 20-132) and

wild turkeys from a nearby, connected flock (21-595 and 21-599) cluster as a clade independent from known *Avibacterium* references.

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Colorado Parks and Wildlife

WILDLIFE RESEARCH PROJECT SUMMARY

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

Period Covered: 1 July 2021–30 June 2022

Principal Investigators: Karen A. Fox, Lance Carpenter

Since 2013, sporadic outbreaks of diarrhea have been observed in mountain goat kids and yearlings in G4. In 2014, this is believed to have contributed to the loss of nearly an entire age class of kids (Figure 1). The cause of mortality in 2013-2014 was suspected to be related to leaking pit toilets and possible exposure to *E. coli*, although reported correction of the issue did not seem to eliminate occurrence of diarrhea. 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 kids and yearlings in G4. The study was repeated in 2021. Fecal samples from marked kids (n=28), their nannies (n=32), and yearlings (n=14) were collected monthly as available during months when the population was accessible by road, primarily June through October. The results from these samples indicated fluctuations in *E. coli* with time, 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. These trends were 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). Results were similar in 2020 and 2021 (Figure 2).

In addition to samples collected from G4, fecal samples from mountain goats in Olympic National Park (ONP) in Washington State were collected and sent to CPW for comparison to G4. The National Park Service was culling this herd, and wildlife health professionals were invited to request samples from these animals. The ONP goats were sampled from 7/20/21-8/3/21 and were not suspected to have contact with people. These adult mountain goats had light to moderate *E. coli* loads. No kids were sampled. The results from ONP subjectively did not seem different from the adult population of G4 goats, although sampling of kids and yearling would be important to specifically compare *E. coli* loads based on age.

Other bacteria results somewhat mirrored the *E. coli* results, suggesting that the gut microflora was generally altered and out of balance from normal when *E. coli* levels were high. 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. Other results included parasite screening which indicated heavier parasitism in ONP than G4, suggesting parasites were not responsible for diarrhea in G4. Electron microscopy detected coronavirus (feces were PCR negative for SARS Co-V-2) in four G4 and adenovirus in one G4 goat. No viruses were detected in the ONP goats. Johne's disease was detected in one G4 goat and no ONP goats.

A definitive cause for diarrhea in G4 was not determined, but *E. coli* remains a concern in the herd, particularly within young animals. Data from this project can serve as a baseline for comparisons if diarrhea re-emerges in the G4 herd and will enable the CPW to refine the management of herd.

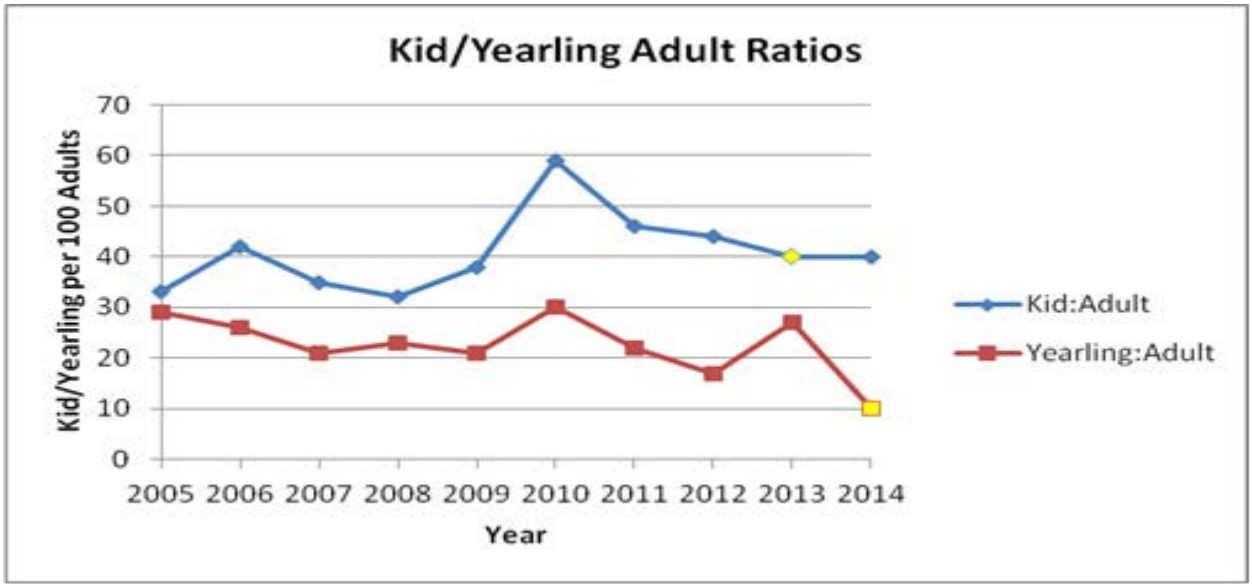

Figure 1. Kid/yearling adult ratios from the Mount Evans summer ground survey 2005-2014. The yellow squares denote the kids' diarrhea outbreak in 2013 with a sharp decline in the yearling/adult ratio in 2014.

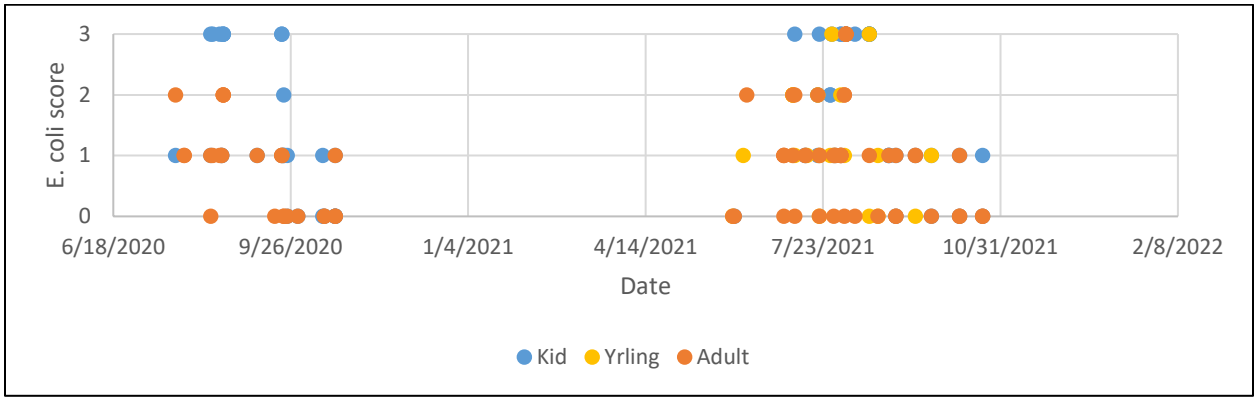

Figure 2. Fecal *E. coli* detections in mountain goats in G4 from 2020 and 2021 samples. 0=no *E. coli* growth on fecal culture; 1=light growth; 2=moderate growth; 3=heavy growth. Note highest *E. coli* loads during the earliest sampling dates in summer, with lower loads in the fall. This could suggest a trend with *E. coli* loads highest during times of highest human activity in the area. This trend is true for kids, yearlings, and adults.

MANAGEMENT AND EDUCATION ACTIVITIES

Veterinary medical activities 2021-2022.

Location of services & primary user	Species	Type of veterinary medical activities
CPW Foothills Wildlife Research Facility (FWRF)	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, and emergency medical care for rehabilitating wildlife housed at FCWF.
Multiple sites statewide; C. Anderson, M. Alldredge, E. Bergman, N. Rayl & collaborators	mule deer, elk, moose	Ultrasounded, sampled, & assessed body condition as part of various field research studies. Provided tailored sedation as well as supportive veterinary care for captured animals.
Statewide; wildlife managers, terrestrial biologists	bighorn sheep	Provided veterinary care, tailored tranquilization, and disease sampling for capture and translocation efforts.
Statewide; wildlife managers, terrestrial biologists	black bear, mountain lion, moose, elk, deer, bighorn sheep, others	Prescribe & track usage of immobilization drugs, recommend & refine dosing instructions, troubleshoot issues related to wildlife immobilization, provide training on proper drug & equipment use.
Statewide; wildlife managers, terrestrial biologists, researchers	Colorado terrestrial wildlife	Provided capture assistance, equipment, field sampling, medical supplies, and training for various monitoring, translocation, & research projects statewide.
Statewide; wildlife managers, terrestrial biologists, researchers	Colorado terrestrial wildlife	Served on Animal Care and Use Committee as attending veterinarian and wildlife health reviewers.
CPW Area 12; terrestrial biologists, ; district & area wildlife managers	Swift fox	Provided veterinary and sampling assistance for collaborative swift fox translocation project for foxes being captured and transported from Colorado to Montana for species restoration.

Training and education provided during 2021-2022.

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

Disease management during 2021-2022

Location of services & primary user	Species	Type of activities
Statewide; species conservation and terrestrial biologists, CPW researchers	Gunnison's and white-tailed prairie dogs, black footed ferrets	Plague vaccine bait manufacture. Produced ~568,000 baits to vaccinate ~8,700 acres
South Park Individual Population Area, conservation and terrestrial biologists	Gunnison's prairie dogs	Vector Control for Plague Management: ~450 acres dusted
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	Colorado terrestrial wildlife	Developed guidance for wildlife rehabilitation in coordination with the Colorado Department of Public Health and the Environment.
Statewide; regulations manager	Cervids	Revised disease testing and importation requirements in CH0 and CH11 for captive cervids.

Laboratory Diagnostics Provided during 2021-2022

Location of services & primary user	Species	Type of Laboratory Activities
CPW Foothills Wildlife Research Facility (FWRF); Statewide; wildlife managers, terrestrial biologists	Mule deer, white-tailed deer, elk, moose	Chronic wasting disease surveillance and monitoring. Facilitated testing of over 7,000 samples for CWD
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Black-tailed prairie dogs, white-tailed prairie dogs, Gunnison's prairie dogs, multiple flea species	Plague testing of tissue samples and flea pools - 46 carcasses - 615 flea pools
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Bighorn sheep	Testing of tissues and swabs for respiratory disease pathogens.
FWRF; Statewide; managers, terrestrial biologists, researchers	Cottontails, jack rabbits, snowshoe hare	RHDV2 testing of tissue and fecal samples - 15 carcasses - 30 fecal samples
FWRF; Statewide; wildlife managers, terrestrial biologists, researchers	Coyotes, wolves	Echinococcus multi-plex testing on fecal samples - 33 wild canid fecal samples
FWRF; Statewide; district & area wildlife managers, terrestrial biologists, researchers	Colorado terrestrial wildlife	Serum and tissue banking. 632 samples added to existing serum and tissue banks.
CPW researchers and CPW animal care and use committee	Colorado terrestrial wildlife	Conducted necropsies for statewide wildlife disease surveillance and to determine cause-specific mortality for various ongoing research projects.

2021 CPW Wildlife Health Program Necropsy Report
(Submissions from Jan - Dec 2021)

Species (#nx cases)	Necropsy Findings	# affected	notes
Avian			
1	Bacterial/Ingluvitis	4+	4 house finches, *E. coli*
2	Bacterial/Conjunctivitis	2	2 house finches, presumptive *M. gallisepticum*
1	Bacterial/Unknown	1	1 blue jay, cranial granuloma
6	Trauma/Electrocution	6	1 golden eagle, 5 bald eagles
1	Trauma/Hit by Car	1	1 bald eagle
1	Trauma/Domestic Dog Predation	1	1 great horned owl
1	Trauma/Electrocution/Lead Exposure	1	1 golden eagle
1	Trauma/Avian Predation	1	1 sharp shinned hawk, also parasitism and emaciation
1	Trauma/Gunshot	1	1 red-tailed hawk, complications from shot to crop
4	Viral/West Nile	14+	1 great horned owl (unconfirmed); 12+ crows; 1 bald eagle
1	Fungal/Aspergillosis	1	1 red-tailed hawk
1	Toxic/Barbiturate	1	1 turkey vulture, scavenged euthanized animal
1	Malnutrition	1	1 snowy owl, also hemoparasites (*Plasmodium*)
1	Congenital	1	1 blue jay, scoliosis
1	Capture mortality	1	1 mallard
33	Disease Surveillance	33	7 wild turkeys: *Mycoplasma synoviae;* 17 domestic pigeons: novel *Avibacterium* species*;* 8 wild turkeys: novel *Avibacterium* species; 1 domestic turkey: novel *Avibacterium* species
10	Apparently healthy	10	10 mallards, euthanized for management
5	Undetermined	18+	18+ common mergansers, possibly toxic
Bat			
17	Disease Surveillance/SARS-CoV-2	17	Multiple species, all negative for SARS-CoV-2
1	Disease Surveillance/*P. destructans*	1	1 canyon bat, negative for white nose syndrome
5	Trauma/Domestic Cat Predation	5	3 big brown bats, 2 little brown bats
1	Starvation/Exposure	1	1 big brown bat
1	Viral/Rabies	1	1 canyon bat

Species (#nx cases)	Necropsy Findings	# affected	notes
1	Ill thrift	1	1 little brown bat, parasitism and bacteremia
1	Undetermined	1	Desiccated, unknown species
Bear			
7	Human Conflict	7	
1	Trauma/Hit by Car	1	
1	Trauma/Foreign body migration	1	Peritonitis
Bighorn Sheep			
35	Disease Surveillance/Hit by Car	35	*Mycoplasma ovipneumoniae* (8); leukotoxigenic *Pasteurellaceae* (13); *Pasteurella multocida* (9); sinus tumor (10); bronchopneumonia (2)
4	Disease Surveillance/Predation	4	
3	Disease Surveillance/Lightning Strike	3	
2	Disease Surveillance/Captive Facility Cull	2	*Pasteurella multocida* (1); sinus tumor (2)
1	Disease Surveillance/Hit by Train	1	
1	Disease Surveillance/Trauma/Fall	1	
1	Disease Surveillance/Capture Mortality	1	
1	Disease Surveillance/Cull for Contact with Domestics	1	Leukotoxigenic *Pasteurellaceae* (1)
1	Disease Surveillance/Hunter Harvest	1	*Mycoplasma ovipneumoniae* (1)
3	Viral/Epizootic Hemorrhagic Disease	3	Leukotoxigenic *Pasteurellaceae* (2)
2	Viral/Bluetongue	2	Leukotoxigenic *Pasteurellaceae* (1); sinus tumor (1)
2	Bacterial/Bronchopneumonia	2	*Mycoplasma ovipneumoniae* (2); leukotoxigenic *Pasteurellaceae* (2); *Pasteurella multocida* (2)
1	Diarrhea/Coccidiosis	1	Fecal sample only
1	Neoplasia	1	Duodenal adenocarcinoma with perforation, captive facility
Bobcat			
1	Dog bites	1	Legal
Chipmunk			
3	Capture mortality	3	

Species (#nx cases)	Necropsy Findings	# affected	notes
Cottontail rabbit			
21	Viral/Rabbit hemorrhagic disease	45+	
3	Trauma/Failed predation	3	Two with bacteremia from infected bite wounds (T. pyogenes)
Species (#nx cases)	**Necropsy Findings**	**# affected**	**notes**
3	Trauma/Blunt trauma	3	
2	Bacterial/Tularemia	2	
2	Bacterial/Salmonellosis	2	
3	Undetermined	3	Sample nondiagnostic, autolysis
Coyote			
1	Pyoderma	1	
1	Nondiagnostic	1	Decomposition
Domestic dog			
1	Species ID	1	Possible wolf skull, identified as domestic dog
Elk			
3	Chronic Wasting Disease/Necropsy Diagnosis	3	
3	Malnutrition/Starvation/Orphaned calf	3	Also broken jaw (1)
2	Nutritional/Vit E Deficiency	2	Heart failure
2	Malnutrition/Winter mortality	2	
1	Nutritional/Acidosis	1	Rumenitis and chronic laminitis
4	Bacterial/Necrobacillosis	4	Hoof rot, penetrating wound (2); necrotic stomatitis (1); liver abscesses with hepatic encephalopathy (1)
4	Bacterial/Pasteurellosis	4	Also winter mortality/malnutrition (1)
1	Bacterial/Brain abscess	1	Skull fracture at base of antler
1	Bacterial/Omphalophlebitis	1	
6	Trauma/Blunt trauma	6	Antlered cow, trauma to frontal bone (1)
1	Trauma/Failed Predation/Bear	1	
1	Trauma/Failed Predation/Coyote	1	
2	Pulmonary Edema/Unknown cause	2	
1	Congenital/Hemimelia	1	
1	Exposure	1	Stuck under a rock
1	Illegal Take	1	

1	Age Determination	1	
Species (#nx cases)	**Necropsy Findings**	**# affected**	**notes**
5	Undetermined	5	Tissues only, no carcass submitted
Fox			
1	Parasitic/Sarcoptic mange	3+	Red fox
1	Undetermined	1	Advanced autolysis, swift fox
1	Trauma/Bite Wounds	1	Also anticoagulant rodenticide exposure, red fox
Fox Squirrel			
3	Gunshot/Air Rifle	3	
1	Predation/Domestic Dog	3	
Jackrabbit			
1	Viral/Rabbit Hemorrhagic Disease	3+	
Marmot			
1	Endocrinopathy	1	Bilaterally symmetrical alopecia
Moose			
2	Parasitic/Elaeophorosis	2	
Mountain Lion			
2	Trauma/Bite Wounds	2	Intraspecific (1); domestic dog (1)
1	Trauma/Hit by Car	1	
1	Trauma/Goring	1	
1	Bacterial/Plague	1	
3	Undetermined	3	Multiple young lions affected over several years, respiratory and enteric signs
2	Apparently healthy	2	Euthanized for unusual behavior (1); euthanized for depredation (1)
Mouse			
1	Toxicity/Rodenticide	1	
Mule Deer			
8	Chronic Wasting Disease/Necropsy Diagnosis	8	Also acidosis (1); also head trauma (1); unconfirmed (1)
1	Chronic Wasting Disease Surveillance/Contact with Domestic Elk	1	CWD negative
3	Malnutrition/Winter Conditions	3	Also MCF (1); also severe tooth wear (1)

3	Malnutrition/Starvation/Orphaned	3	Also dog bite wounds (1)
Species (#nx cases)	**Necropsy Findings**	**# affected**	**notes**
2	Ill-Thrift/Fawns	2	Poor condition, parasitism, tissues only submitted
8	Viral/Adenoviral hemorrhagic disease	11	
7	Viral/Bluetongue	11	BTV-6
1	Viral/Malignant Catarrhal Fever	1	
1	Viral/Epizootic Hemorrhagic Disease	1	EHDV-6
2	Bacterial/Necrobacillosis	2	Lymph node abscesses negative for TB surveillance (1); lung abscesses (1)
2	Bacterial/Bronchopneumonia	2	*B. trehalosi* (1); *T. pyogenes* (1); *Mycoplasma* species unconfirmed (2)
1	Bacterial/Lymphadenitis	1	*T. pyogenes, P.multocida*, negative for TB surveillance
1	Bacterial/Pinkeye	10+	Submitted case also had bronchopneumonia; *Moraxella, Streptococcus, E. coli*
1	Parasitism/Toxoplasmosis	1	Unconfirmed
1	Parasitism/Demodectic Mange	1	
2	Congenital/Contracted Tendons	2	Suspect intrauterine trauma
1	Congenital/Stillborn/Suspect Dystocia	1	
1	Congenital/Anterior Segment Dysgenesis	1	
2	Trauma/Undetermined	2	
2	Trauma/Hit by Car	2	
3	Legal/ Poaching	5	One case involved doe and two fetuses (gunshot to uterus)
2	Vaginitis, mild	2	Opportunistic examination from VIT
1	Human Conflict, Euthanized	1	Suspected habituation
1	Neoplasia	1	Metastatic intestinal adenocarcinoma
1	Normal	1	Meat submitted, no lesions observed (normal tendon)
1	Undetermined	1	Tissues only, no necropsy
Pine Marten			
1	Capture mortality	1	
Prairie Dog			
1	Bacterial/Plague	1	
1	Undetermined	1	Hemorrhagic pneumonia, possibly related to *Mycoplasma* sp.

1	Predation/Avian	1	
Species (#nx cases)	**Necropsy Findings**	**# affected**	**notes**
1	Predation/Bobcat	1	
1	Predation/Undetermined	1	
1	Capture mortality	1	Exposure
1	Venous thrombosis	1	Infarcted colon
1	Nondiagnostic	1	Decomposition
2	Trauma	2	Head trauma (1); Hit by car (1)
Pronghorn			
2	Undetermined	2	Possible tetany (1); myocardial necrosis (2); suspect plant toxicity
Raccoon			
2	Viral/Canine Distemper	2	
Red Squirrel			
1	Capture Mortality	1	
White-tailed Deer			
3	Chronic Wasting Disease/Necropsy Diagnosis	3	also supplemental feeding (1); also acidosis (1)

WILDLIFE HEALTH PROGRAM PUBLISHED, IN PRESS, & IN REVIEW MANUSCRIPTS DURING 2021-2022

Published FY 2021-22

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