Testing the Tools: Montana's Highlands Bighorn Sheep [*Ovis canadensis*] Project

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ABSTRACT: More than a dozen of Montana's bighorn sheep (*Ovis canadensis*) herds have experienced all-age pneumonia die-offs in the past two decades and most have yet to fully recover. Wildlife managers have employed various strategies to help restore these herds such as natural herd re-establishment (hands-off approach), augmentations, and complete herd removal. Using the Highlands bighorn sheep herd in SW Montana, we designed a 5-year study to explore the efficacy of a tool for restoring bighorn sheep herds following a pneumonia outbreak: identification of and subsequent removal of chronic shedders of *Mycoplasma ovipneumoniae* (*M. ovi*). Utilizing the metapopulation structure of the Highlands herd, we will collect two years of baseline information on the five sub-herds that comprise the Highlands metapopulation to 1) monitor disease exposure of individuals and identify chronic shedders, 2) monitor lamb survival, and 3) estimate connectivity of sub-herds. We will then implement two years of a testing and removal of known chronic shedders across all five sub-herds. The efficacy of this treatment will be monitored through year 5. A decrease in prevalence of *M. ovi* shedding and exposure in the sub-herds would indicate success of the management tool. Results of this experiment will add to the growing knowledge of test and removal as a viable tool to manage struggling bighorn sheep herds across Montana and the intermountain West.

Biennial Symposium of the Northern Wild Sheep and Goat Council 23:35-39; 2022

KEYWORDS: Bighorn sheep, chronic shedder, *Mycoplasma ovipneumonia*, *Ovis canadensis, test and removal*

INTRODUCTION

Bighorn sheep herd recovery in Montana (MT) following disease-related die-off events has had mixed success. Currently, Montana Fish, Wildlife and Parks (FWP) is exploring a novel approach to facilitate and expedite herd recovery. In some populations, bighorn sheep augmentations have failed to improve herd performance following all-age pneumonia die-off's. Such is the case in the Highland Mountain area where bighorn sheep experienced a die-off in 1994-95. Historically, bighorn sheep in this area were extirpated in the early 1900's and re-established by a transplant from Sun River in 1967. The population increased to 300-400 animals over the ensuing decades until the 1994-95 die-off reduced the population by 90%. Since then, multiple augmentations from five MT populations (Sun River, Bonner, Sula, Ruby Mountains, Fergus) added 118 sheep to the Highlands herd. The last augmentation occurred in 2015. Despite these efforts, the Highlands population continues to struggle with low population growth.

In the Highlands population and several others in MT, population recovery following an all-age pneumonia event is suspected to be limited by persistent disease. Bighorn sheep populations commonly host respiratory pathogens that may reduce recruitment rates for some time post die-off (Butler et al. 2017, 2018).

Due to testing protocols and interpretations that do not account for uncertainty in pathogen detection, respiratory disease dynamics are poorly understood; however, it is believed to be a polymicrobial disease that is initiated by *Mycoplasma ovipneumoniae* (M.ovi) that permits other bacterial species, such as those in the *Pasteurellaceae* family, to invade the lungs through inhibition of ciliary action (Cassirer et al. 2018). The initial outcome of M.ovi infection is often all-age die-offs resulting in high levels of mortality (Cassirer et al. 2018). These initial die-offs are commonly followed by annual epizootics among juveniles and sporadic pneumonia mortality among adults (Cassirer et al. 2013, Smith et al. 2014). Additionally, environmental and individual factors such as use of mineral licks, nutritional status, social interactions (Manlove et al. 2014), and movement behaviors (Lowrey et al. 2020) may interact with pathogens to influence disease expression. Trace mineral deficiencies may also lead to immunological deficiencies and increase susceptibility to bacterial infections (Flueck et al. 2012, Garrott et al. 2020), as well as physiological stress (Ayotte et al. 2006). Despite these uncertainties in the drivers of respiratory disease, our current focus is on reducing transmission of M.ovi.

Ground observations from 2007-2015 confirmed that the Highlands bighorn sheep population is comprised of five sub-herds (Foothills, LaMarche, Notch Bottom, Red Mountain, Sheep Mountain). Ewes appear to display sub-herd fidelity while rams frequently move between sub-herds; however, there is some overlap in ewe home ranges between at least two of the sub-herds. The bighorn sheep population objective for the Highlands Mountains (Hunting District 340) is a minimum viable population of 125 animals (FWP Conservation Strategy, 2010). The current estimated abundance is approximately 120 animals and has exhibited a stable population for the past several years.

The objective of this project is to evaluate the efficacy of test and removal management actions to increase lamb survival and population performance, and ultimately inform management to recover struggling bighorn sheep populations. In some bighorn sheep populations, removal of chronically infected individuals from herds has been shown to potentially improve population performance (Garwood et al. 2020). However, it can be difficult to identify individuals who are chronically shedding the pathogen because the gregarious nature of bighorn sheep within their ewe-lamb groups makes the likelihood of M.ovi transmission high, resulting multiple individuals being intermittently infected despite not being chronic shedders. The presence of these intermittent shedders makes it difficult to identify chronic shedders in the absence of serial testing. Additionally, given the difficulties in repeat capturing bighorn sheep for disease testing, uncertainty in disease testing efficacy (Butler et al. 2017, Paterson et al. 2020), repeat testing may or may not accurately identify all chronically infected individuals. It also is not currently known if the removal of all chronically shedding individuals is required to affect the desired changes in population performance. Therefore, there is a need to critically evaluate whether the identification and removal of chronic shedders can affect the desired management outcomes.

STUDY AREA

The Highlands Hunting District 340, located just south of Butte, contains approximately 1,141 square miles and includes the Highland Mountains and the northern portion of the East Pioneer Mountains near the town of Melrose. Interstate 15 and the Big Hole River separate the two mountain chains. The district is comprised of shrub-grasslands (sagebrush, mountain mahogany, bluebunch wheatgrass, Idaho fescue), coniferous forests, and agricultural lands. Forty-two percent of the district is in private ownership located primarily at the lower elevations of the district. The majority of private land is in agricultural production, primarily cattle although there are several hobby sheep and goat farms as well.

Approximately 233 square miles of the district (20%) is currently occupied by bighorn sheep during some portion of the year. Sixteen percent of the occupied area is private land and 84% is public lands. Bighorn sheep winter range comprises approximately 188 square miles of this district (16%); 23% is private land and 77% public lands, with the majority of public land being administered by the BLM. Based on past and current telemetry data and recent observations, the majority of the bighorn sheep population winters on public lands.

The vegetation within the occupied bighorn sheep range is predominantly rocky terrain interspersed with sagebrush-grassland, mountain mahogany, and lodgepole-Douglas fir forest. Elevation ranges from 1,460-3,100 meters. Annual precipitation ranges from 20-30 cm at the lowest elevations to 53-79 cm at the upper elevations.

METHODS/FUTURE PLANS

Our overall experimental design was to evaluate the effectiveness of test and removal on lamb survival and population performance in a before-after treatment design. All five sub herds within the Highland Mountains will be monitored for two years prior to management treatments. This monitoring period is required to determine sub-herd disease exposure, chronic shedders (those individuals with 2 or more positive exposures to M.ovi), monitor baseline lamb survival, and identify potential interchange among sub-herds. Management treatments will be implemented in years 3 and 4 of the project and monitored through year 5. This monitoring period is required to determine post-treatment pathogen exposure and lamb survival. At the conclusion of this study, based on results of the treatment, management actions may be continued or modified. If no post-treatment improvement in lamb survival and population performance is observed and disease is confirmed within the treated sub-herds, the sub-herds with persistent disease exposure may be removed.

In all sub-herds, we propose to capture, collect biological samples and GPS collar all ewes, capture sample and GPS collar as many 6-month lambs as possible, and capture, sample and GPS collar up to 20 males per year. Ewes will be outfitted with a vaginal implant transmitter to locate her lamb for neonatal capture and collaring. The data from collared ewes will be used to delineate seasonal ranges, document movements of lamb-ewe groups and identify potential interchange between sub-herds. The data from collared rams will be used to document the extent and types of movements between groups, assuming that rams are the demographic group most likely transmitting pathogens among sub-herds in the metapopulation. Neonatal lambs will be outfitted with GPS or VHF expandable collars for tracking their survival to age 1.

Some captures may be possible with drop netting, ground darting or trapping, and helicopter net-gunning will be used for remaining capture operations. Sampling will include disease testing to screen for pathogen shedding, collection of blood for serology and pregnancy assessment (ewe's only), and a body condition assessment. The pathogen testing protocol will include: 1) collecting blood for serological testing for exposure to M.ovi and a suite of viral pathogens including CE, PI3, BRSV, OPP, IBR, and a trace mineral assessment, 2) collecting pellets to screen for lungworm and intestinal parasites, 3) collecting replicate nasal swabs to test for M.ovi using PCR, and 4) collecting replicate tonsil swabs for aerobic bacterial culture and testing for Leukotoxin A using PCR. Each animal each year will be classified as infected if either the first or the second nasal swab is positive for exposure to *M.ovi*. Given the difficulty of repeatedly capturing and sampling every ewe every year and uncertainty in testing results, conclusive evidence for differences in pathogen communities between sub-herds and identifying chronically infected animals may be difficult or impossible to evaluate. Nonetheless, we plan to utilize replicate swabs and a combination of aerial and ground capture efforts to maximize sample sizes and make sampling as rigorous as possible. Whole blood will also be fixed on a gene card and the eartag punch saved for genetic archiving.

Animals with at least 2 repeated positive M.ovi PCR tests will be classified as chronically infected and animals with a single positive M.ovi test will be considered infected. In this management experiment, we plan to test individuals 1-2 times per year and remove any individuals that are chronically shedding M. ovi. We will euthanize any chronically shedding bighorn sheep during capture. Any chronic shedders identified post release through conventional pathogen testing methodologies will be radio tracked and euthanized with a gunshot. All heads will be collected and examined for nasal tumors.

We will monitor lamb survival using two approaches: first, we will estimate the number of lambs produced, the number surviving until the end of summer and the number surviving until the end of spring using a combination of ground and aerial counts and classifications; and second, we will estimate lamb survival directly based on a sampling of marked individuals. We will capture lambs as neonates and outfit each with a VHF or GPS collar that remotely monitors live/dead status. Lambs will be monitored for survival and observed for signs of pneumonia infection 3-4 times per week. Causes of mortality will be investigated. During each observation, the group composition and size will be recorded. The presence of a marked ewe's live lamb will be determined based on nursing, body contact or other association patterns (see Cassirer et al. 2013), or the lamb's collar. This information will be used to document the interchange and spatial patterns of lamb-ewe groups during the lamb rearing period, estimate annual lamb survival for each sub-herd, and document the presence of disease and/or infection.

ACKNOWLEDGEMENTS

The authors would like to thank Wild Sheep Foundation, Montana Wild Sheep Foundation, Montana FWP, and the Northern Wild Sheep and Goat Council for their support and encouragement of this project. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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