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Evaluating Bighorn Sheep Herd Response After Selective Removal of *Mycoplasma ovipneumoniae* Chronic Shedders

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EVALUATING BIGHORN SHEEP HERD RESPONSE AFTER SELECTIVE REMOVAL OF

MYCOPLASMA OVIPNEUMONIAE CHRONIC SHEDDERS

BY

TYLER J. GARWOOD

A thesis submitted in partial fulfillment of the requirements for the

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2018

EVALUATING BIGHORN SHEEP HERD RESPONSE AFTER SELECTIVE REMOVAL OF

MYCOPLASMA OVIPNEUMONIAE CHRONIC SHEDDERS

TYLER J. GARWOOD

This thesis is approved as a creditable and independent investigation by a candidate

for the Master of Science in Wildlife and Fisheries Sciences degree and is acceptable

for meeting the thesis requirements for this degree. Acceptance of this thesis does

not imply that the conclusions reached by the candidate are necessarily the

conclusions of the major department.

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REMOVAL OF *MYCOPLASMA OVIPNEUMONIAE* CHRONIC SHEDDERS

TYLER GARWOOD

2018

Infected individuals vary in their contribution to disease persistence, and
chronically infected individuals may sustain disease in a population. One disease
that might persist in a population through chronically infected individuals is
pneumonia in wild sheep. *Mycoplasma ovipneumoniae* (*Mo*), a pathogen of Caprinae
commonly present in domestic sheep and goats, strongly correlates with pneumonia
epizootics when it infects wild sheep populations. These epizootics can cause 40-
100% herd mortality in an initial all-age dieoff, precipitate annual lamb mortality as
high as 100% in following years, and sustain adult mortality long after initial all-age
dieoffs. We conducted an experiment in the Black Hills of South Dakota to evaluate
whether we could eliminate *Mo* infection and pneumonia in a bighorn sheep
population by removing chronically infected individuals, termed "chronic shedders".
We classified chronic shedders as adults that consistently tested positive for *Mo* on
multiple nasal swabs collected over a 20 month period. We identified and removed
chronic shedders from a treatment population (Custer State Park) and left the
adjacent control population (Rapid City) unmanipulated. *Mo* and respiratory disease
were not detected following treatment, whereas *Mo* persisted in the control herd
and pneumonia was the leading source of mortality among both adults and
lambs. Adult and juvenile annual survival in the treatment population averaged 93%

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and 76%, respectively, as compared to 83% and 35% in the control herd. Overall
mortality hazard for adults was significantly reduced in the treatment population
relative to the control ($\beta_{treatment}$ =-0.95, CI=-2.03, -0.039), as was the hazard for
lambs ($\beta_{treatment}$=-1.40, CI=-2.42, -0.46). These results support the hypothesis that
Mo is the primary causative agent of epizootics of pneumonia in bighorn sheep, are a
proof-of-concept of epidemics being sustained by chronic carriage, and provide
direction for management actions aimed at treating respiratory disease in bighorn
sheep.

CHAPTER 1: REMOVAL OF BIGHORN SHEEP CHRONICALLY INFECTED WITH *MYCOPLASMA OVIPNEUMONIAE* ELIMINATES PATHOGEN PRESENCE AND PNEUMONIA IN POPULATION, PROVIDING EVIDENCE OF CHRONIC CARRIERS SUSTAINING DISEASE TRANSMISSION

Abstract

Infected individuals vary in their contribution to disease persistence, and chronically infected individuals may sustain disease in a population. One disease that might persist in a population through chronically infected individuals is pneumonia in wild sheep. *Mycoplasma ovipneumoniae* (*Mo*), a pathogen of Caprinae commonly present in domestic sheep and goats, is associated with pneumonia epidemics when it infects wild sheep populations. These infections linger and are responsible for adult mortality long after initial all-age dieoffs. We conducted an experiment in the Black Hills of South Dakota to evaluate whether we could eliminate *Mo* infection and pneumonia in a bighorn sheep population by identifying and removing individuals that chronically carry and shed *Mo*, termed "chronic shedders". We classified chronic shedders as adults that consistently tested positive for *Mo* on multiple nasal swabs collected over a 20 month period. We identified and removed chronic shedders from a treatment population (Custer State Park) and left the adjacent control population (Rapid City) unmanipulated. *Mo* and respiratory disease were not detected following treatment, whereas *Mo* persisted in the control herd and pneumonia was the leading source of mortality among adults. Adult survival in the treatment population averaged 93% annually, as compared to 83% in the control herd. Overall mortality hazard for adults was significantly reduced in the treatment population relative to the control herd ($\beta_{treatment}$ =-0.95, CI=-2.03, -0.039). This outcome supports the hypothesis that *Mo* is a primary agent of pneumonia infections in bighorn sheep and is maintained by chronic shedders in free-ranging populations. These results provide direction for management actions

aimed at treating respiratory disease in bighorn sheep and are a proof-of-concept of disease being sustained in a population by chronic carriage.

Introduction

Variation among individuals in their contribution to disease spread and persistence has been theorized to serve an important role in sustaining disease presence in a population (Woolhouse et al. 1997, Lloyd-Smith et al. 2005, Paull et al. 2012). One way individuals vary is in their propensity to chronically sustain infections, and it has been suggested that a few chronically infected individuals can sustain the presence certain diseases by shedding the responsible pathogen and reinfecting other individuals within their population (Monack et al. 2004, Buhnerkempe et al. 2017). To our knowledge, experimental evidence of disease presence being eliminated by identifying and isolating such "chronic shedders" remains undocumented in the literature, despite the fact that chronic shedding may be ubiquitous across a variety of taxa (Wertheim et al. 2005, Buhnerkempe et al. 2017, Plowright et al. 2017). Experimental evidence testing the concept of eliminating chronic shedders to control disease outbreaks would demonstrate the efficacy of this disease management action and be applicable across the human, domestic animal, and wildlife health sectors.

Epizootics of pneumonia have generally precipitated significant declines in bighorn sheep (*Ovis canadensis*) populations across the American West (Valdez and Krausman 1999, Singer et al. 2000). Researchers also noted that pneumonia is often hyperendemic in post-epizootic populations, preventing population recovery (Cassirer and Sinclair 2007, Smith et al. 2015). Evidence suggests that domestic

Caprinae serve as a reservoir for the responsible infectious agent (Monello et al. 2001, George et al. 2008, Besser et al. 2013, Besser et al. 2017), and that pathogen transmission can occur when bighorns interact with domestic sheep and goats (George et al. 2008). Infected bighorns also infect naïve bighorns, causing intraspecies transmission within and between herds (Cassirer et al. 2013). After subsequent transmission within the bighorn population, an all-age epizootic can occur, and may initially kill 40-80% of the herd (Spraker et al. 1984, Enk et al. 2001). The all-age epizootic is often followed by annual juvenile pneumonia epizootics of similar or greater magnitude (Cassirer et al. 2013, Smith et al. 2014). Adults may also experience pneumonia-related mortalities in years following the initial all-age dieoff (Smith et al. 2015). Because of the importance of adult bighorn sheep in maintaining high population growth rates (Johnson et al. 2010) and their likely role in contributing to juvenile pneumonia epizootics (Plowright et al. 2017), treating and preventing adult pneumonia could be an important component to ensuring population recovery in ailing herds.

Historical management attempts to curtail pneumonia mortality rates in wild bighorn sheep populations have been varied and directed at a wide variety of causal agents, but met with little success. Antibiotics (Coggins and Matthews 1998, Rudolph et al. 2007, McAdoo et al. 2010), vaccination (Cassirer et al. 2001, Sirochman et al. 2012), partial and complete depopulation (Cassirer et al. 1996, McFarlane and Aoude 2010, Bernatowicz et al. 2016), and mineral supplementation (Coggins 2006) have all been tested. One likely reason for the unsuccessful results is that the techniques targeted the wrong etiological agent. Several bacteria (i.e.,

Pasteurella multocida, *Bibersteinia trehalosi, Mannheimia haemolytica*) and parasites (*Protostongylus* sp) are detected in the lungs of bighorn sheep fatally affected by pneumonia and have been the target of numerous treatments (Foreyt et al. 1994, Miller et al. 2000, Dassanayake et al. 2009, Grigg et al. 2017)*. Some researchers still identify leukotoxigenic *Pasteurella* as playing the primary role in pneumonia development (Dassanayake et al. 2017, Grigg et al. 2017). However, of all potential candidates, bacterial *Mycoplasma ovipneumoniae* (*Mo*) exhibits the strongest correlation with bighorn sheep pneumonia mortalities (Besser et al. 2012, Besser et al. 2013, Cassirer et al. 2018), a trend that was discovered only a decade ago with access to improved molecular techniques (Besser et al. 2008). Ultimately, the lack of a clearly successful treatment indicates that more research is needed to develop management solutions, and that definitively identifying the causative agent of pneumonia in bighorn sheep would allow wildlife managers and veterinarians to better direct their limited resources (Cassirer et al. 2018).

Years of studying pneumonic bighorn sheep herds have produced useful epidemiological insights that suggest further research avenues. One such insight is that ewe infection is likely responsible for annual juvenile epizootics; most juvenile pneumonia mortalities occur prior to weaning, and lambs rarely interact with individuals outside their nursery groups during this timeframe (Cassirer et al. 2013, Manlove et al. 2017). Whether ewe-driven transmission plays a strong role in persistent adult pneumonia mortalities is less clear, but it is possible given that pneumonia-induced mortalities generally occur during and shortly after rut when close contact between sexes might increase transmission (Bleich et al. 1997,

Cassirer et al. 2013). Secondly, only a small proportion (median=22%) of bighorns tend to test positive for shedding *Mo* in a given herd at any point in time, despite high herd seroprevalence (median =67%; Cassirer et al. 2018). Although only based on a single sub-population, a third paper noted that 50% of bighorns remained positive for *Mo* infection after their first positive test, suggesting chronic carriage in some individuals (Plowright et al. 2017). Based on this groundwork, it is reasonable to hypothesize that *Mo* may be maintained in bighorn sheep populations by chronic shedder ewes that are few in number and identifiable with adequate testing intensity. A natural prediction of this hypothesis is that identifying and removing all chronically shedding ewes would reduce or eliminate pneumonia in that herd and increase survival. Since chronic shedders may maintain disease within a population in a variety of taxa (Foley et al. 1997, Wertheim et al. 2005, Buhnerkempe et al. 2017), research designed to test this hypothesis could yield insights applicable beyond bighorn sheep management.

To test the effect of chronic shedder removal, we utilized free-ranging bighorn sheep herds near Custer State Park (CSP; treatment herd) and Rapid City (control herd), South Dakota, USA (Figure 1). Our objectives were to 1) determine if removing chronically shedding ewes would reduce the prevalence of *Mo* shedding in the treatment herd, and 2) document whether the chronic shedder removal would be accompanied by a lack of pneumonia-related mortality in adult bighorn sheep.

Methods

Study Area

The Black Hills are a small, isolated mountain range rising from the Great Plains of southwestern South Dakota and east-central Wyoming (Froiland 1990) that occupy an area of approximately 8,400 square kilometers (Fecske et al. 2004). They range in elevation from 972 meters above sea level to 2,207 meters at Black Elk Peak. Ponderosa pine (*Pinus ponderosa*) is the dominant tree species (Brown and Sieg 2016). Black Hills spruce (*Picea glauca* Densata) and aspen (*Populus tremuloides*) increase in abundance at higher elevations in the central and northern Black Hills. Based on data collected at the Rapid City Airport weather station, average annual precipitation over the course of our study was 11.3 cm of rainfall and 29.6 cm of snow. Temperatures ranged from -30°C to 41°C, with an average high of 17°C and an average low of 1°C (National Oceanic and Atmospheric Administration 2018).

Bighorn sheep were likely common in the Black Hills before European settlement but extirpated by 1899 (Seton 1929, Witte and Gallager 2012). Beginning in 1922, managers and conservationists performed 9 reintroduction events that resulted in current populations of bighorn sheep in 5 distinct locations: CSP, Rapid City, Elk Mountain, Hell Canyon, Deadwood (South Dakota Department of Game Fish and Parks 2018). Only the CSP and Rapid City herds were known to be experiencing pneumonia epizootics at the time of chronic shedder removal and *Mo* presence was verified in both (Smith et al. 2015) (Table 1). These herds utilized ranges that were spatially isolated by approximately 12 km straight-line distance (Figure 1). We did not observe range overlap during the course of the study. Both areas were easily accessible by U.S. Forest Service and state fire roads. Predator assemblages were similar between study areas and consisted of mountain lions (*Puma concolor*),

coyotes (*Canis latrans*), bobcats (*Lynx rufus*), bald eagles (*Haliaeetus leucocephalus*), and golden eagles (*Aquila chrysaetos*) (Smith et al. 2015).

We designated the CSP herd as the treatment herd, which was located primarily within a 28,733 ha park in Custer County, South Dakota. Source herds for the treatment herd were Whiskey Mountain, Wyoming (22 bighorns, 1965) and Alberta, Canada (20 bighorns, 1999)(South Dakota Department of Game Fish and Parks 2018). Deep canyons and exposed rocky outcroppings in the central and northeastern regions of the park characterized bighorn sheep habitat in the treatment area. At the beginning of the study, the treatment population numbered 14 ewes, 6 rams, and 2 lambs. No farms inside this study area were known to raise domestic sheep or goats, but several residents kept domestic sheep within 10 km of the park boundary.

We designated the Rapid City herd as the control herd, which inhabited a mixture of public and private land in Spring Creek and Rapid Creek canyons near Rapid City in Pennington County, South Dakota. Control source herds were Georgetown, Colorado (26 bighorns, 1991) and Badlands National Park, South Dakota (5 bighorns, 1992) (South Dakota Department of Game Fish and Parks 2018). Bighorn sheep in the control herd generally used canyon bottoms and walls for parturition and summer range, and then moved to residential lawns closer to Rapid City for winter range (Smith et al. 2014, Smith et al. 2015). At least one farm within the control study area kept domestic sheep and goats. Approximately 45 ewes, 20 rams, and 5 lambs inhabited in the control area at the beginning of the study.

Radio-collaring, Data Collection, and Pathogen Detection

We chemically immobilized bighorn sheep (BAM; 0.43 mg/kg butorphanol, 0.29 mg/kg, azaperone, 0.17 mg/kg medetomidine, Wildlife Pharmaceuticals) via dart rifle (Dan-Inject, Børkop, Denmark, EU) or captured them by net-gunning from a helicopter (Quicksilver Air, Inc., Fairbanks, AK and Hells Canyon Helicopters Lewiston, ID). We fitted bighorn sheep with very high frequency (VHF) collars (M252OB; ATS) to detect mortality events and facilitate repeated sampling. We also attached color-coded, numbered tags to the collar and ear to enable individual identification.

We collected information and samples regarding sex, age, *Mo* carriage/antibody presence, and the presence of other pathogens during capture. We examined molar and incisor eruption on the lower jaw to estimate age, which allowed us to reliably age individuals up to 3.5 years old (Valdez and Krausman 1999). Adults older than 3.5 were treated as a single group. We collected *Mo* mucosal samples via three nasal swabs, which were consecutively inserted deep into each of the nares and then removed while being slowly rotated around the wall of the nasal cavity (Drew et al. 2014). Two of these swabs were returned to their sheath and one was immersed in a Tryptic Soy Broth media with 15% Glycerol (Hardy Diagnostics; Butler et al. 2017). To detect *Mo* antibodies, we collected blood, from which we obtained serum. To detect the presence of other pneumonia-inducing bacteria, we rotated oropharyngeal swabs along the back of the throat while ensuring that the swab contacted each tonsilar crypt, and then stored them similarly to nasal swabs. We refrigerated all swabs and serum and then shipped

them to the Washington Animal Disease Diagnostic Lab (WADDL), or Dr. Thomas

Besser's lab at Washington State University (Pullman, Washington), for analyses.

To detect *Mo* presence and to estimate its abundance in a nasal swab sample,

we extracted and amplified bacterial deoxyribonucleic acid (DNA) using realtime

polymerase chain reaction (RT-PCR) techniques (McAuliffe et al. 2003, Besser et al.

2008). We deemed a sample to be positive if fluorescence generation exceeded the

threshold before the 36th RT-PCR cycle, indeterminate if detected between the 36th

and 40th cycle, and negative if undetected through all 40 cycles. Utilizing the

collected serum, we determined *Mo* antibody presence by competitive enzyme-

linked immunosorbent assay (ELISA) using standard techniques (Ziegler et al.

2014). We documented the presence of other pathogens on nasal and

oropharyngeal swabs through culture and PCR techniques customarily performed

on swab samples from bighorn sheep (Besser et al. 2008).

Chronic Shedder Identification and Experimental Removal

We commenced *Mo* testing of adult bighorn sheep in the treatment herd in August

2014 and compiled *Mo* histories for each individual in that herd by April 2016. We

strove to sample each animal for *Mo* presence 3 times before April but obtained a

minimum of 2 tests on every adult individual before or shortly after experimental

manipulation (Table 1). When we compiled all tests for a given individual, we

classified them as a chronic shedder (always tested positive after the first positive

test), intermittent shedder (negative test after positive test), or non-shedder (all

negative tests). We repeated this process for all individuals in the treatment herd

prior to experimental manipulation. We also documented the *Mo* shedding status of

control herd sheep to gauge baseline shedding rates and facilitate future

management actions (Table 2).

Once we identified chronic shedders in the treatment herd, we relocated

them to a penned facility at South Dakota State University (SDSU). Post-removal, we

monitored adult bighorn sheep in both the treatment and control herd for *Mo*

presence using similar testing techniques. The South Dakota State University

Institutional Animal Care and Use Committee approved all capture and handling

procedures prior to project initiation (Approval number 16-00A). We developed

capture protocol based on recommendations from the American Society of

Mammalogists (Sikes and Animal Care and Use Committee of the American Society

of Mammalogists 2016).

Survival Monitoring

To obtain high-quality cause-specific mortality data on adult bighorn sheep, we

needed to investigate and collect cadavers in a timely fashion. The collars we

deployed were movement-sensitive and changed signal transmission from 40 to 80

pulses/min when the collar was not moved for ≥8 hours. Hence, we monitored for

mortality signals from the collars of the adults a minimum of 2 times a week in both

study areas using handheld directional antennas from 22 January 2016 to 1 May

2018. We also attempted to observe all adults when possible because predators and

scavengers may move collars as they consume carcasses, delaying mortality

detection and thereby making a cause-of-death determination difficult.

Upon detecting a mortality signal from the collar, we immediately located the

bighorn and assessed the site for evidence indicative of potential causes of death.

Specifically, we examined the site for signs of predator presence (feces, tracks, scrapes; Elbroch 2003) and the bighorn carcass for caching, bite marks, hemorrhaging, and skeletal disarticulation (Stonehouse et al. 2016). We also noted if the cadavers were generally still intact, which provided evidence of potential pneumonia or other health-related causes. We performed field necropsies of intact cadavers, with an emphasis on investigating the respiratory tract. If the cadaver was relatively fresh, we collected nasal swabs and the sheep's lungs and shipped them to WADDL for pathogen testing. We used the test results to supplement field observations in assigning the likelihood of the various causes of death. For all samples, we incorporated our knowledge based on field necropsies, evidence at the site, and previous behavioral observations of the individual to assign probabilities to each cause-of-death category (Table 3). Cause-specific mortality categories were "predation", "pneumonia", "human-caused", and "other". This created a vector of probabilities across all categories, and these probabilities summed to one. When cause of death was certain, we created the vector by assigning a single, non-zero entry to the appropriate cause-of-death category. Based on the causes of death that were assigned as most likely in the probability vectors, as well as the date of those deaths, we determined mean dates for each cause-specific mortality source and used ANOVA to determine whether the differences between mean dates were statistically significant.

Survival Analysis

We deemed treatment (i.e., herd identity), testing positive for *Mo* bacteria, year of the study (Gaillard et al. 2000), individual sex (Jorgenson et al. 1997), and age

(Loison et al. 1999) as covariates potentially important in affecting survival of adult bighorn sheep. We coded the treatment effect as a constant binary variable throughout the study, assigning individuals in the treatment herd to "1" and those in the control herd to "0". We also coded the positive test effect as a constant binary variable, assigning individuals that tested positive at least once to "1" and those that never tested positive to "0". Similarly, we coded sex as a constant binary variable, giving males a value of "1" and females "0". We considered age time-varying and treated it as a categorical variable separated into 4 groups based on an individuals age class (1-2 years old, 2-3 years old, 3-4 years old, and 4+ years old). Individuals advanced in age class on May 22nd each year to correspond with peak lambing time in our study area. Year was a categorical variable and spanned 2016–2018. We treated it as a time-varying covariate and assigned each week of the study to the appropriate calendar year.

To incorporate covariates, we built *a priori* models that tested relevant hypotheses about the disease ecology of bighorn sheep in the Black Hills. In our global model (Model 8, Table 4), we calculated log unit cumulative hazard as

$$\ln(\Lambda_{i,j}) = \gamma +$$

$$\beta_{treatment} \times treatment_i + \beta_{positive\ test} \times positive\ test_i + \beta_{year}\ [\ year_{ij}] +$$

$\beta_{age}\ [age_{ij}] + \beta_{sex} \times sex_i + \rho_j$, where γ was the base-line, log unit cumulative hazard rate. We denoted the effect of the treatment as $\beta_{treatment}$, with $treatment_i$ being an indicator for the treatment herd. We similarly designated the effect of a positive test as $\beta_{positive\ test}$, with $positive\ test_i$ being an indicator for an individual with at least one positive test. We assigned β_{year} as the effect of year, with $\beta_{year}\ [1]$

indicating the effect of 2017 and β_{year} [2] indicating the effect of 2018 on the ith

individual in the jth week. We denoted β_{age} as the effect of an individual's age,

with β_{age} [1] indicating the effect of being 2-3 years old, β_{age}[2] indicating the effect

of being 3-4 years old, and β_{age}[3] indicating the effect of being 4 or more years old.

We signified the effect of an individual's sex through β_{sex}, with sex_i being an

indicator of males. We signified the week effect with ρ_j.

Using a novel Bayesian time-to-event survival analysis framework

implemented through Nimble in Program R, we fit the models to the collected data

(NIMBLE Development Team 2018, Walsh et al. 2018). This framework first

calculated the overall weekly hazard of dying irrespective of cause of death (Cross et

al. 2015) using a weakly informative truncated-normal prior on the baseline log unit

cumulative hazard that assumed a mean annual adult survival of 86% and a 95%

probability of lying in the interval of ~20% to ~ 95%

($\gamma \sim \text{dnorm}[-3.745, \text{precision} = 3.5]$ T$[-9.5, -2]$; all priors are specified in BUGS

language format; Loison et al. 1999). We specified an intrinsically conditional

autoregressive prior (ICAR; Heisey 2010, Cressie and Wikle 2011) for the effect of

each week on the overall hazard (ρ_j) to account for variability and temporal

correlation in the weekly hazard rates. Thus, we specified a prior with a uniform

distribution ($\rho_1 \sim dunif(-0.5, \text{precision} = 0.5)$) for the first week effect, and the

effect for the jth week was specified as $\rho_j \sim \text{dnorm}(\rho_{j-1}, \text{precision} = \tau)$. Lastly, we

specified the prior for the precision parameter as: ($\tau \sim \text{dgamma}(1, \text{precision} = 1)$;

Heisey 2010). The ICAR prior provided temporal smoothing across weekly hazard

estimates. Priors on covariate effects were flat ($\beta_x = \text{dnorm}(0, \text{precision} = 0.01)$).

In the second component of our framework, we calculated cause-specific mortality by extending Cross et al.'s (2015) methodology to explicitly incorporate observer uncertainty into parameter estimation (Walsh et al. 2018). Specifically, we treated the true cause of death for each individual as a latent unknown variable for which we assigned a vector of prior predictive probabilities. These prior predictive probabilities specified the observer's belief that each cause of death of interest was the true cause of death given their assessment of the death site evidence (Table 3). We then imputed the true cause of death using a data augmentation approach that generated a cause of death at each Markov chain Monte Carlo (MCMC) iteration based on a categorical distribution with a parameter vector equal to the prior predictive probability vector specified for that individual. Using random starting values, we ran 3 MCMC chains for 100,000 iterations with the first 10,000 repetitions removed for burn-in.

We calculated Watanabe-Akaike Information Criteria (WAIC) from each model after running our 7 *a priori* models and compared them to identify the models that best described the data (Table 4; Gelman et al. 2014). We considered models that differed by ≤ 2 WAIC as potential alternatives to the selected model with the caveat that we preferred a more parsimonious model (Burnham and Anderson 2002, Arnold 2010). Therefore, we based our conclusions on the parameter estimates from the most parsimonious model with the lowest WAIC value. We calculated 95% credible intervals (CI) for all estimated parameters.

Results

Overall Capture and Testing Effort

We radio-collared 92 bighorns between 1 August 2014 and 1 May 2018: 33 were in the treatment herd and 59 were in the control herd. By the study's conclusion, we were monitoring 100% of the treatment herd and approximately 90% of the control herd.

Chronic Shedder Testing and Removal

We tested 24 bighorn sheep for *Mo* in the treatment herd prior to finalizing chronic shedder removal: 7 males and 17 females (Table 1). This accounted for all bighorn sheep known to be present in the treatment study area. We tested each bighorn sheep 1 (n=2), 2 (n=10), 3 (n=10), or 4 (n=2) times for *Mo* presence over 60 samples. Fifty-one (85%) of these samples tested negative, 1 (2%) tested indeterminate, and 8 (13%) tested positive. Two female individuals produced 7 of the 8 positive tests and always tested positive for *Mo*. We classified these bighorns as chronically shedding *Mo* and removed them from the population on 13 March 2016 (Table 1). A ewe that later tested negative on her second test and died before chronic shedder removal generated the other positive test. One other ewe died before chronic shedder removal, leaving 20 individuals present immediately post-removal. We concluded testing to identify chronic shedders on 8 April 2016.

Post-Removal Pathogen Testing in Treatment Herd

During the first 2 years following chronic shedder removal, we tested 26 individuals in the treatment herd: 9 males and 17 females (Table 2). This accounted for all adult bighorn sheep in the area, as 9 individuals were recruited into the population and 3 died before they could be retested following chronic shedder removal. We tested each sheep 1 (n=17) or 2 (n=9) times for *Mo* presence over the course of 35 tests.

We did not detect *Mo* post-removal in the treatment herd, as 26 (74%) of the tests were negative and 9 (26%) were indeterminate. Ten (50%) individuals had detectable antibodies, 8 (40%) did not have detectable antibodies, and 2 (10%) were indeterminate for antibodies. We did not obtain serum for 6 individuals in the treatment herd. Other potential pneumonia agents we detected after chronic shedder removal were *Trueperella pyogenes* (n=4, 15% of treatment herd), *Bibersteinia trehalosi* (n=8, 31% of treatment herd), *Mannheimia* sp. (n=4, 15% of treatment herd), *Mannheimia haemolytica* (n=1, 4% of treatment herd), and *Pasteurella* sp. (n=1, 4% of treatment herd).

Pathogen Testing in Control Herd

Between 1 January 2016 and 1 May 2018, we tested 58 different bighorn sheep in the control herd: 18 males and 40 females (Table 5). We tested each sheep 1 (n=38), 2 (n=18), or 3 (n=2) times for *Mo* presence over 80 tests. We detected *Mo* in 38 tests (48%). Nine were indeterminate (11%) and 33 were negative (41%). Forty-nine (94%) of the tested bighorns in the control herd had detectable antibodies, 1 (2%) had an indeterminate antibody status, and 2 (4%) had no detectable antibodies. We also detected *Trueperella pyogenes* (n=25, 43% of control herd), *Bibersteinia trehalosi* (n=23, 40% of control herd), *Mannheimia* sp. (n=2, 3% of control herd), *Mannheimia haemolytica* (n=1, 2% of control herd), *Mannheimia glucosida* (n=1, 2% of control herd)*,* and leukotoxigenic *Pasteurella* (n=1, 2% of control herd) in the control herd.

Mortalities and Removals

We documented 24 mortalities in both study populations (5 treatment, 19 control) following chronic shedder removal in the treatment herd. We censored 2 mortalities in the control herd that were related to capture. Of the 22 adult bighorn mortalities that occurred from causes not related to capture, we documented 5 (2 males, 3 females) in the treatment herd and 17 (6 males, 11 females) in the control herd. These mortalities constituted 29% of adult bighorn sheep in the control herd and 17% in the treatment herd. Using the most likely cause of death based on field evidence (see Table 3 for full assigned cause-specific probabilities), we determined that 80% of mortalities were due to predation events (n=4) and the remaining 20% were due to other causes (n=1) in the treatment herd (Figure 2[i]). In the control herd, we attributed 59% of mortalities to pneumonia (n=10), 23% to human causes (n=4), 12% to other causes (n=2), and 6% to predatory events (n=1) (Figure 2[i]). Across study areas, we calculated the peak week for pneumonia death as 20-27 November 2016 (sd=6 weeks), for predation as 14-20 August 2016 (sd=8 weeks), for human-causes as 27 November-3 December 2017 (sd=14 weeks), and for other causes as August 27-September 2, 2017 (sd=8 weeks) (Figure 3). We did not find these peaks to be statistically distinguishable at the 95% confidence level (p=0.0773).

Survival Analysis

We included 86 bighorn sheep in the survival analysis. Our analysis included 29 bighorns (10 rams, 19 ewes) in the treatment herd and 57 bighorns (19 rams, 38 ewes) in the control herd. We commenced the analysis with 13 March 2016 and concluded it with 1 May 2018, encompassing 112 weeks of data. We included 22

mortalities (described in the previous paragraph) in the analysis. As categorized by age at the end of the study, we followed 16 (9 in treatment, 7 in control) 1-2 year olds, 4 (2 in treatment, 2 in control) 2-3 year olds, 10 (4 in treatment, 6 in control) 3-4 year olds, and 56 (14 in treatment, 42 in control) bighorns that were 4 years of age or older.

According to WAIC values (w_i =0.61), we found that our data most strongly supported the following model: $ln(\Lambda_{ij}) = \gamma + \beta_{treatment} \times treatment_i + \rho_j$. No other model was within 2 ΔWAIC (Model 1, Table 4). Hence, we calculated and reported the log unit cumulative hazard measurements for each week based on this model (Figure 3). Our selected model suggested that living in a herd where chronic shedders were removed had a negative mean effect on overall adult weekly cumulative hazard ($\beta_{treatment}$ =-0.95) that was statistically significant (CI=-2.03, -0.039). This corresponds with an annual adult survival rate of 93% (CI=76%, 98%) in the treatment herd and 83% (CI=75%, 89%) in the control herd.

We calculated that the hazard of dying from pneumonia for adults in the treatment herd was significantly reduced relative to the control herd (Probability difference = -51%, CI=-78%, -15%)(Figure 2[ii]). Adult bighorns in the treatment herd had a 10% probability of dying from pneumonia (CI=0%, 41%) and control herd adults had a 61% probability of dying from pneumonia (CI=36%, 84%). Conversely, adult bighorn sheep in the treatment herd were significantly more likely to be killed by predators than those in the control herd (treatment herd probability= 61%, CI=22%, 92%; control herd probability=10%, CI=1%, 27%; probability difference=51%, CI=13%, 83%). We attributed all known predation mortalities to

mountain lions. The probabilities of dying from other causes and human-induced mortality events were similar between study areas.

Although other models were not as strongly supported as Model 1, they provided insight on how other effects may have contributed to an individual's hazard. Among effects other than $\beta_{treatment}$, only β_{year} was found to be statistically significant, with 2017 and 2018 having lower hazard than 2016 ($\beta_{year}[2017] = -2.32$, CI=-4.19, -0.68; $\beta_{year}[2018] = -3.75$, CI= -7.99, -0.08; Model 4, Figure 4). We did not find β_{age}, $\beta_{positive\ test}$, and β_{sex} to be significant in the most strongly supported models that included those effects (Model 6, $\beta_{age}[2-3]$= -0.29, CI= -1.85, 1.00, $\beta_{age}[3-4]$= -0.69, CI=-2.61, 0.82, $\beta_{age}[4+]$= -0.60, CI=-1.45, 0.25; Model 3, $\beta_{positive\ test}$= -0.52, CI=-1.33, 0.25; Model 2, β_{sex}= -0.01, CI=-0.87, 0.78).

Discussion

Pneumonia and *Mo* infection were no longer detected in the treatment herd after the removal of chronic shedders (Table 5, Figure 2[i]). Our findings provide strong evidence that *Mo* chronic shedders play a critical role in pneumonia persistence in bighorn sheep, and that removal of chronic shedders can be used as a management tool in ailing bighorn sheep herds. A formal experimental structure such as the one presented here is rare in free-range, large mammal studies and allows for stronger inferences about the effect of chronic shedder removal than any published study to date. Our ability to monitor every bighorn sheep in our treatment herd also allowed for definitive statements about cause-specific adult mortality sources, which is an area of speculation in many observational wildlife studies (Cassirer and Sinclair 2007, Smith et al. 2015).

Predation rates on adult bighorn sheep were significantly higher in the treatment herd, which may be explained by the unique movement patterns of the control herd. Previous studies suggest that mountain lion predation on bighorns increases during the winter and early spring (Ross et al. 1997, Hayes et al. 2000, Cassirer and Sinclair 2007). Our control herd inhabited wintering grounds in the suburban neighborhoods of Rapid City during this season of potentially increased predation pressure. Although we lack fine-scale predator density data for this study area, predators have been shown in previous studies to preferentially avoid urban/suburban areas (Rebolo-Ifran et al. 2017, Biel et al. 2018). For treatment herd sheep, urban habitat was unavailable within their home range, potentially making them more vulnerable to predation. Ultimately, the treatment herd's survival rates were higher than other healthy herds, indicating that predation did not strongly impact the treatment herd survival rate.

We included age class in our model based on its important role in describing survival in previous research (Jorgenson et al. 1997, Loison et al. 1999), but we did not find this effect to be significant in the populations we studied. Jorgenson et al. (1997) found survival to be high among 2-7 year old bighorn sheep, only detecting potential senescence at ages older than 7. Unfortunately, we were not able to reliably age individuals older than 4 years, so the effect of senescence was likely undetectable in that age class because of the wide range of ages included. Jorgenson et al. (1997) also found yearlings to have lower survival than prime-aged adults, a finding that is common among ungulates (Clutton-Brock and Albon 1982, Gaillard et al. 2000). That yearlings in our study did not have significantly lower survival than

other age classes is more surprising, because we were able to specifically age these individuals (n= 30 yearlings). Similarly, sex was not a significant determinate of hazard in the populations we studied, whereas Jorgenson et al. (1997) found a significant sex effect. Our lack of a sex effect may be explained by the relatively low hunting pressure in our study areas; only one sheep (male) was harvested in either of our study areas over the course of the experiment, whereas Jorgenson et al. (1997) noted that hunting accounted for significant mortality among rams older than 5 years (also see Festa-Bianchet 1989).

Researchers first hypothesized that *Mo* was a necessary pathogenic component driving bighorn sheep pneumonia epizootics nearly a decade ago (Besser et al. 2008), and our study convincingly supports this hypothesis. Despite the polymicrobial nature of bighorn sheep pneumonia, our removal efforts were focused solely on those individuals carrying *Mo* (Table 1)*.* Samples taken from individuals not removed in the treatment herd revealed a suite of other pathogens associated with respiratory disease, including *Bibersteinia trehalosi, Mannheimia haemolytica* (lktA positive and negative), and *Trueperella pyogenes* (Tables 1 and 5). However, their presence failed to induce pneumonia in treatment herd adults. This finding makes physiological sense: while other pathogens clearly contribute to disease manifestation (Besser et al. 2013), *Mo* appears to be necessary, through its effect of disrupting mucociliary clearance, for these other pathogens to establish lung infections (Niang et al. 1998, Cassirer et al. 2018). Our findings therefore suggest that *Mo* should be the focal pathogen of management actions aimed at eliminating respiratory disease infections in bighorn sheep herds.

We initially speculated that three sampling events per individual over a 16-20 month period was an appropriate sampling intensity to identify chronic shedders with a minimum of 4-6 months between tests (Table 1). Previous work supports this sampling frequency, reporting that the vast majority (93%) of intermittent shedders returned to non-shedding status by their third test or within 1 - 2 years of their first positive sample (Plowright et al. 2017). Based on data collected in our treatment herd, we found that a single test would have been sufficient to identify chronic shedders. Conversely, we observed 9 bighorns in the control herd that tested PCR-negative for *Mo* on their first test and positive on their second test. If we were testing each control herd individual once before removal, these individuals would be left in the population despite potentially later changing to a chronic shedders. We only observed one individual (2% of the control population) switch from PCR-positive to PCR-negative in the control herd, indicating that a policy of removing individuals immediately after a single test positive may be a cost effective way of identifying chronic shedders in the control herd while minimizing unnecessary removals. In other herds, removal after a single PCR-positive test could result in higher removal of non-chronic shedders, as Plowright et al. (2017) observed 12 individuals (28% of all individuals tested) that returned to non-shedding states after a positive test. However, if a herd can sustain higher removal rates, classifying chronic shedders based on a single positive test may be a cost-effective method for removing pneumonia from the population. Another option could be developing a "culture rule" for *Mo* that differentiates between persistent and intermittent or non-carriers across populations with high reliability using a minimal number of tests.

Such techniques have been successfully developed to identify *Staphylococcus aureus* chronic carriers among humans (Nouwen et al. 2004), and would provide more clarity on appropriate testing intensity.

Reduced adult mortality following chronic shedder removal is a novel and encouraging finding. In previous studies, adult survival rates in healthy and pneumonic bighorn herds tended to converge after the initial all-age epizootic and, in some cases, were higher than before disease introduction (Manlove et al. 2016). However, comparing survival rates over a wide variety of time frames and locations makes it difficult to discern the effect of pneumonia on adult survival across studies. The close proximity of our study areas allowed us to make a reasonable assumption that climatic, nutritional, and other important factors were similar when making comparisons between treatment and control herd vital rates. Therefore, we reasonably speculate that pneumonia has an additive effect on adult mortality in our study area, since overall mortality hazard in the non-pneumonic treatment herd was significantly lower over the course of our study.

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Table 1: Testing dates and results for bighorn sheep sampled in the treatment herd prior to and shortly after chronic shedder removal, 1 August 2014- 8 April 2016. Individuals who always tested positive after their first positive test were considered chronic shedders, and removed 13 March 2016. Antibody presence indicates exposure to *Mo*.

Treatment Identifier	Sex	Age	PCR Test Result[1]				Chronic Shedder	*Mo* Antibodies[2]	Other Detected Pathogens[3]	Test Dates
			1	2	3	4				
1	♀	4+	-	-	-	-	No	D	*Bt*	8/1/14, 1/1/15, 10/22/15, 3/1/16
2	♀	4+	+	+	+	+	Yes	D	*Bt*	8/6/14, 4/15/15, 10/22/15, 3/13/16
3	♀	4+	-	-	-		No	D	*Bt*	8/11/14, 11/16/15, 3/13/16
4	♀	4+	-	-	-		No	D		8/26/14, 10/31/14, 9/9/2015
5	♂	4+	-	-	-		No	D	*Bt, Tp*	1/15/15, 12/3/15, 3/13/16
6	♂	4+	-	-	in		No	D		1/15/15, 11/5/15, 2/11/16
7	♀	4+	-	-	-		No	D	*LktA*	1/28/2015, 11/4/15, 3/1/16
8	♀	4+	-	-	-		No	D		1/15/15, 9/15/15, 2/29/16
9	♀	4+	-	-	-		No	D		1/15/15, 10/2/15, 3/7/16
10	♀	0.5	-	-			No	D	*Bt, Tp*	3/20/15, 4/8/16
11	♀	0.5	-	-			No	D	*Bt, Tp*	3/24/15, 4/8/16
12	♀	1.5	-	-	-		No	D	*Bt*	4/6/15, 9/11/15, 3/8/16
13	♂	1.5	-	-			No	ND	*Ms*	4/7/15, 4/8/16
14	♂	0.5	-	-			No	ND	*Bt, Tp, Ms*	4/7/15, 3/13/16
15	♂	2.5	-	-			No	ND	*Bt*	7/28/15, 3/13/16
16	♂	3.5	-	-			No	ND	*Bt*	7/28/15, 3/13/16
17	♀	4+	-	-			No	D		7/30/15, 4/8/16
18	♀	4+	-	-			No	D	*Bt, Tp*	9/3/15, 3/13/16
19	♀	4+	+	+	+		Yes	D		10/20/14, 10/31/15, 3/13/16
20	♀	4+	-	-	-		No	D	*Tp, LktA*	2/4/15, 4/6/15, 2/22/16
21	♂	0.5	-				No	in		4/4/16
22	♀	0.5	-				No	ND	*Bt, LktA*	4/4/16
23	♀	4+	+	-			No	D		8/26/14, 5/1/15
24	♀	3.5	-	-			No	D		3/19/15, 10/20/15

[1] - = Not Detected, + = Detected, in = Indeterminate ; [2] D = Detected, ND = Not Detected, in = Indeterminate; [3] *Bt* = Bibersteinia *trehalosi*, *Tp* = Trueperella *pyogenes*, *LktA* = Leukotoxigenic *Pasteurella*, *Ms = Mannheimia* sp

Table 2: Testing dates and results for bighorns sampled in the control herd over the course of the study, 1 January 2016- 1 May 2018. Antibody presence indicates exposure to *Mo*.

Control Identifier	Sex	PCR Test Result[1]			Chronic Shedder	*Mo* Antibodies[2]	Other Pathogens Detected[3]	Test Dates
		1	2	3				
1	♂	+			No	D	*Lkta*	4/6/16
2	♂	+	-		No	D	*Tp*	3/11/16, 2/5/17
3	♀	-	-		No	D	*Tp, Ms*	3/11/16, 2/5/17
4	♀	-	in		No	D	*Bt, Tp*	3/12/16, 2/4/17
5	♂	-	+		No	D	*Tp*	3/22/16, 2/7/17
6	♀	-	in		No	D	*Tp*	3/22/16, 2/5/17
7	♀	-	-		No	D	*Bt, Tp, Mg*	3/22/16, 2/5/17
8	♀	in	in		No	D	*Bt,Tp, Mh*	3/22/16, 2/5/17
9	♂	-			No	D		3/22/16
10	♂	+			No	D		3/22/16
11	♀	+			No	D		3/22/16
12	♂	+	+		Yes	D	*Tp*	3/22/16, 2/7/17
13	♂	+			No	NT		10/26/16
14	♀	-			No	D		11/16/16
15	♀	+			No	D	*Bt*	11/17/16
16	♂	-			No	D	*Tp*	2/5/17
17	♀	in	+		No	D	*Tp*	3/11/16, 2/5/17
18	♀	+	+	+	Yes	D	*Tp*	3/11/16, 2/5/17, 4/17/18
19	♀	-	+	in	No	D	*Bt, Tp*	3/13/16, 2/7/17, 4/6/17
20	♀	-			No	D	*Bt,Tp*	3/13/16
21	♂	-			No	NT		3/22/16
22	♀	-	+		No	D	*Tp*	3/22/16, 2/7/17
23	♀	-	+		No	D	*Bt, Tp*	3/22/16, 2/7/17
24	♀	-	+		No	D	*Bt, Tp*	3/22/16, 2/7/17
25	♀	+	+		Yes	D	*Bt, Tp*	2/23/16, 2/7/17
26	♂	+			No	NT		10/6/16
27	♂	+			No	D	*Bt, Tp*	12/13/16
28	♀	+			No	D	*Tp*	12/20/16

#	Sex	Col1	Col2			Species	Dates
29	♀	-	+	No	D	Bt, Tp	2/27/16, 2/7/17
30	♂	-		No	D	Bt	2/7/17
31	♂	+		No	NT		2/7/17
32	♀	-		No	D	Tp	2/7/17
33	♀	in		No	D		2/7/17
34	♀	-	+	No	D	Bt, Ms	3/7/16, 2/7/17
35	♀	-		No	NT		2/2/18
36	♀	-	+	No	D	Bt, Tp	3/8/16, 2/7/17
37	♀	-	+	No	D	Bt	3/9/16, 4/13/17
38	♂	-		No	D	Bt	3/13/16
39	♀	-	-	No	D	Tp	3/13/16, 2/7/17
40	♀	+		No	D		3/13/16
41	♀	-		No	ND		1/22/16
42	♀	+		No	D	Bt	1/22/16
43	♂	+		No	D	Bt	1/27/16
44	♀	in		No	D	Bt, Tp	1/27/16
45	♀	+		No	ND	Bt	2/18/16
46	♀	+		No	D	Bt	2/18/16
47	♀	+		No	D	Bt, Tp	2/27/16
48	♀	-		No	D	Bt	3/8/16
49	♀	-		No	D		3/11/16
50	♀	-		No	D		3/11/16
51	♀	+		No	D		3/11/16
52	♂	+		No	in		3/11/16
53	♂	+		No	NT		3/11/16
54	♂	-		No	D		3/11/16
55	♀	+		No	D		3/11/16
56	♀	-		No	D		3/11/16
57	♀	in		No	D		3/11/16
58	♀	+		No	D		3/12/16

[1] - = Not Detected, + = Detected, in = Indeterminate; [2] D = Detected, ND = Not Detected, NT= Not Tested, in = Indeterminate ; [3] Lkta = Leukotoxigenic *Pasteurella, Tp = Trueperella pyogenes, Ms= Mannheimia sp, Bt= Bibersteinia trehalosi, Ma= Mannheimia glucosida, Mh=Mannheimia haemolytica*

Table 3: Cause of death probabilities assigned to all individuals that were included in the survival analysis. For analysis, these probabilities were converted into vectors that summed to one across cause-specific categories for each individual that died.

Treatment Identifier	Human-Caused	Other	Pneumonia	Predation
1				100%
3		5%	5%	90%
6				100%
7		100%		
14				100%

Control Identifier	Human Caused	Other	Pneumonia	Predation
1		40%	60%	
9	100%			
12		40%	60%	
20		5%	95%	
21			100%	
24		90%		10%
28	25%		75%	
41	100%			
42	100%			
43		10%	90%	
45	100%			
46			25%	75%
47	25%		75%	
50			100%	
51		70%	30%	
53		10%	90%	
56		5%	95%	

Table 4: Descriptions and rankings of all models tested to explain log unit cumulative hazard $[ln(\Lambda_{ij})]$ for each individual adult bighorn sheep, 13 March 2016 – 1 May 2018. Ranking is based upon Watanabe-Akaike Information Criteria (WAIC) and is reported with ΔWAIC (difference in WAIC between top model and model being compared) and w_i (WAIC weight). γ is baseline log unit cumulative hazard rate, $\beta_{treatment}$ is the effect of the treatment (whether an individual was in the treatment or control herd), β_{year} is the effect of year, β_{age} is the effect of age, β_{sex} is the effect of sex, and ρ_j is the effect of a given week (j) with a random walk prior for temporal smoothing across estimates.

Model Description	Effects	WAIC	ΔWAIC	w_i
1) Hazard varied by treatment	γ , $\beta_{treatment}$, ρ_j	344.7	0	0.61
2) Hazard varied by treatment and sex	γ , $\beta_{treatment}$, β_{sex} , ρ_j	346.9	2.2	0.20
3) Hazard varied by treatment and positive test	γ , $\beta_{treatment}$, $\beta_{positive\ test}$, ρ_j	348.3	3.6	0.10
4) Hazard varied by treatment and year	γ , $\beta_{treatment}$, β_{year} , ρ_j	349.5	4.8	0.05
5) Hazard varied by treatment, year, and sex	γ , $\beta_{treatment}$, β_{year} , β_{sex} , ρ_j	351.5	6.8	0.02
6) Hazard varied by treatment and age	γ , $\beta_{treatment}$, β_{age} , ρ_j	354.1	9.4	0.01
7) Hazard varied by treatment, age, and sex	γ , $\beta_{treatment}$, β_{age} , β_{sex} , ρ_j	357.5	12.8	0.00
8) Global Model	γ , $\beta_{treatment}$, β_{year} , β_{age} , β_{sex} $\beta_{positive\ test}$, ρ_j	359.8	15.1	0.00

Table 5: Testing frequency, results, and dates for bighorns sampled in the treatment herd after chronic shedder removal, 9 April 2016 - 1 May 2018. Antibody presence indicates past exposure to *Mo*.

Treatment Identifier	PCR Test Result[1] 1	PCR Test Result[1] 2	Chronic Shedder	*Mo* Antibodies[2]	Other Pathogens Detected[3]	Test Dates
4	-		No	D	*Tp*	2/4/17
5	-	-	No	D	*Bt, Tp, Ms*	11/10/16, 2/4/17
6	-	in	No	NT	*Tp*	11/28/16, 2/8/17
7	-		No	in		2/4/17
8	-		No	ND		2/4/17
9	-	in	No	ND	*Ms, Mh*	11/1/16, 2/4/17
10	-		No	D		2/6/17
11	-	-	No	D	*Bt*	10/15/16, 2/4/17
12	-		No	ND	*Bt, Tp*	2/6/17
13	-		No	NT		2/6/17
15	in	-	No	D		8/5/16, 2/8/17
16	in		No	D	*Ms*	2/4/17
17	-		No	NT		2/4/17
18	-		No	D		2/4/17
20	-		No	D		10/16/16, 2/4/17
21	-		No	in		2/4/17
22	-		No	ND		2/4/17
25	in		No	ND	*Bt, Ms*	2/8/17
26	-		No	NT	*Bt*	2/6/17
27	-		No	NT		2/6/17
28	-	in	No	ND		8/8/16, 2/8/17
29	-	in	No	ND		8/8/16, 2/8/17
30	in	in	No	ND	*Bt*	9/20/16, 2/4/17
31	-	-	No	D	*Bt*	9/21/16, 2/4/17
32	-		No	NT	*Ps*	2/6/17
33	-		No	D	*Bt*	9/18/17

[1] - = Not Detected, + = Detected, in = Indeterminate; [2] D = Detected, ND = Not Detected, NT= Not Tested, in = Indeterminate ; [3] *Tp = Trueperella pyogenes, Bt = Bibersteinia trehalosi, Ms = Mannheimia sp, Mh=Mannheimia haemolytica, Ps = Pastuerella sp.*

Figure 1: Ranges of study populations of bighorn sheep in the Black Hills, South Dakota, USA, 2016-2018. The Custer State Park herd is the treatment herd.
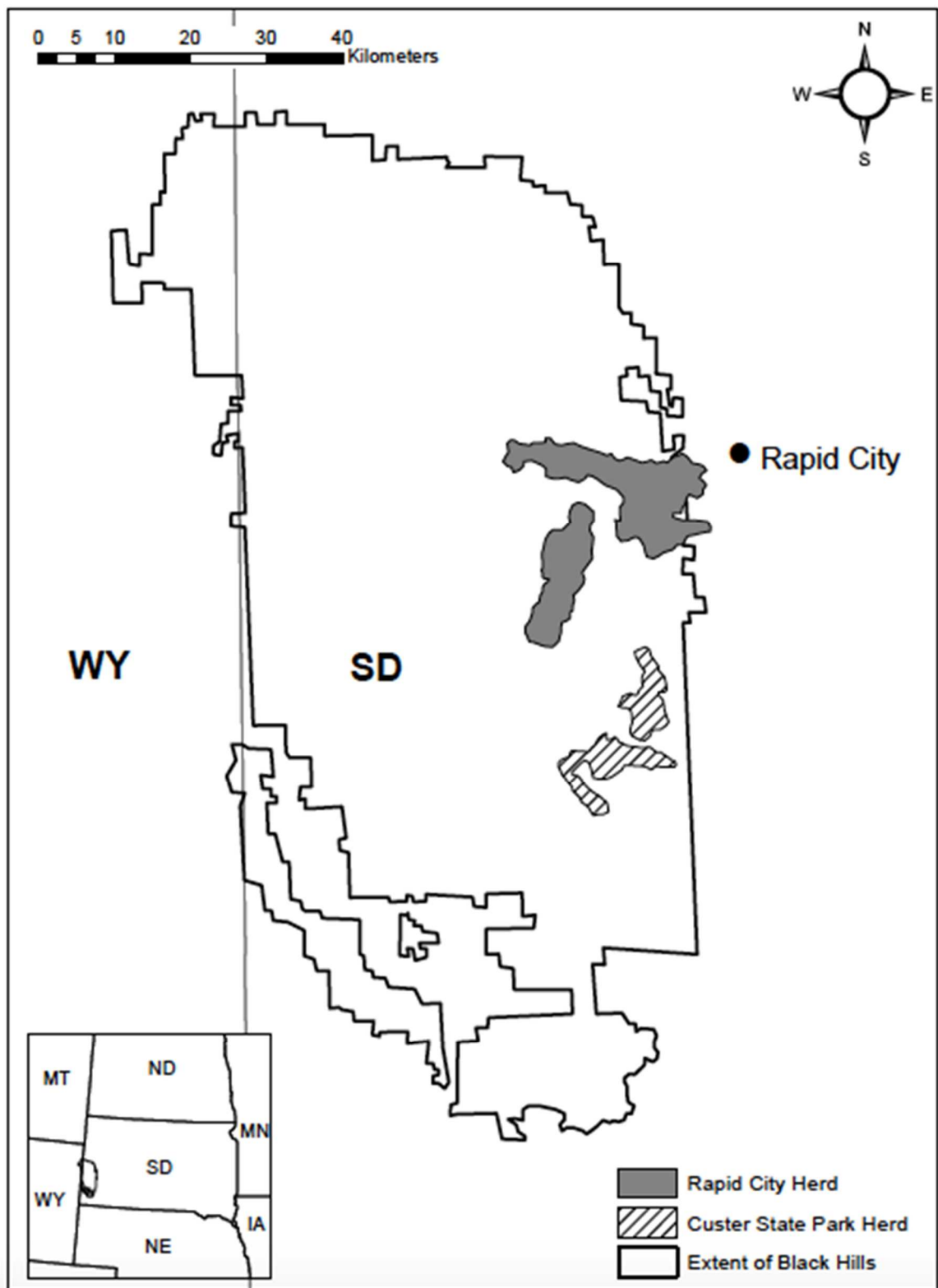
Figure 2: A comparison of mortality sources for adult bighorn sheep between herds after chronic shedder removal in the treatment herd on 13 March 2016. Panel [i] shows cause-specific mortality sources based on the most likely cause of death assigned in the field. No pneumonia was recorded in the treatment herd during the study. Panel [ii] shows the estimated cause-specific mortality probabilities based on the survival analysis. It is significantly less likely that bighorn sheep will die from pneumonia in the treatment herd, but more likely that they will die from predation. Credible intervals are 95%.
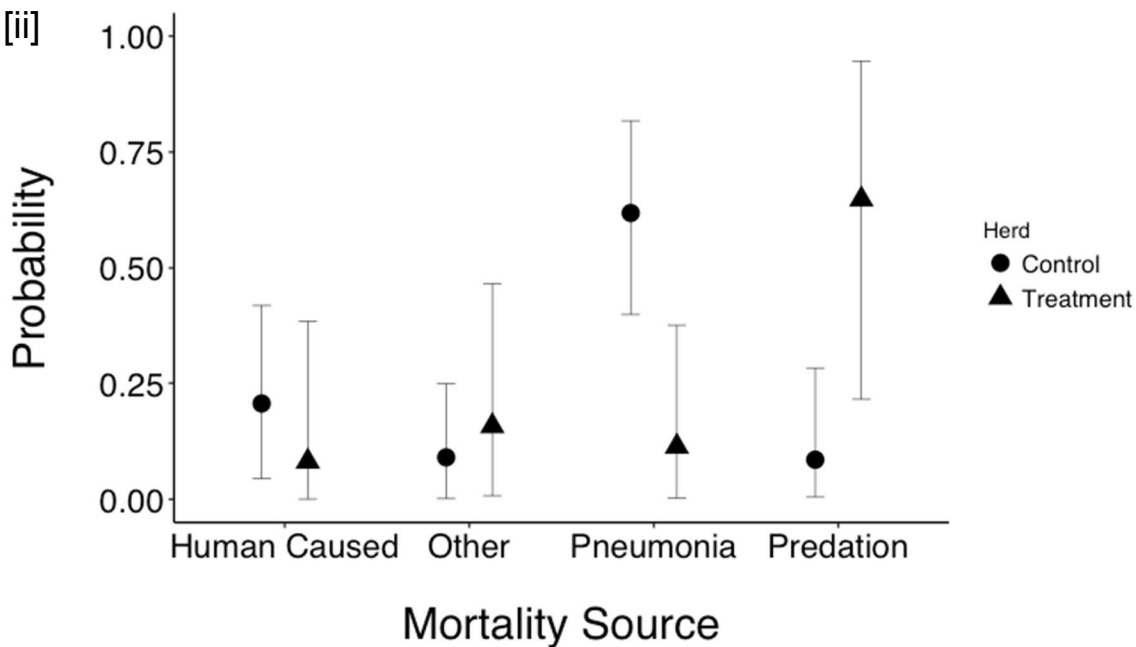
[i]

[ii]
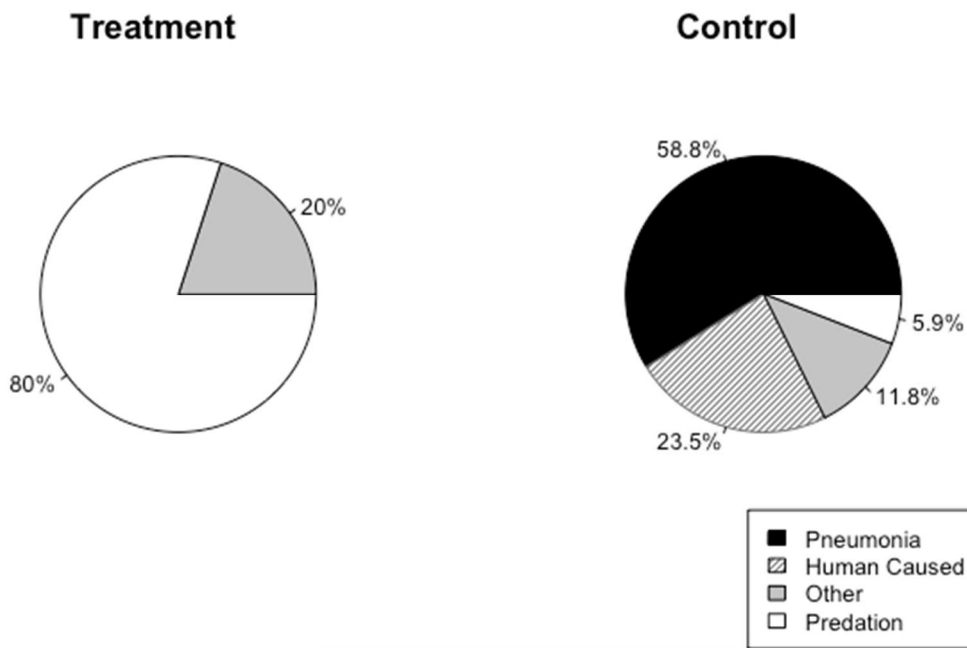
Figure 3: Overall log hazard for an adult bighorn sheep in Rapid City (γ), 13 March 2016-1 May 2018. Plot is based on our top model, $ln(\Lambda_{ij}) = \gamma + \beta_{treatment}$ x $treatment_i + \rho_j$, where $ln(\Lambda_{ij})$ is the unit log cumulative hazard for the ith individual in the jth week, $\beta_{treatment}$ is the effect of being in the treatment herd, $treatment_i$ denotes whether individual i was in the treatment herd, and ρ_j is the effect of a given week (j) which is temporally smoothed via a conditional intrinsic autoregressive random walk prior. Brackets indicate peak periods for each cause specific mortality source, and 95% credible intervals are shown in gray.
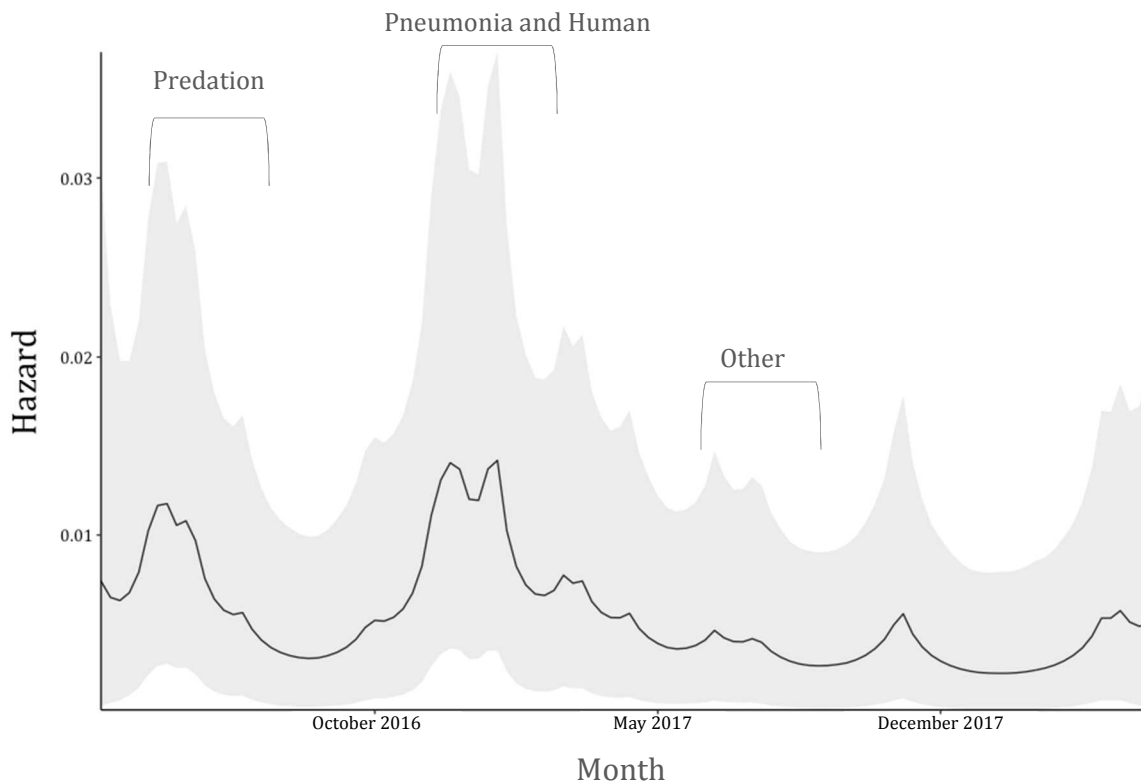
CHAPTER 2: *MYCOPLASMA OVIPNEUMONIAE* CHRONIC SHEDDER REMOVAL IS

ASSOCIATED WITH IMPROVED HEALTH AND SURVIVAL OF BIGHORN SHEEP

LAMBS

This chapter is being prepared for publication and was coauthored by Daniel P. Walsh,

E. Frances Cassirer, Thomas E. Besser, Chadwick P. Lehman, and Jonathan A. Jenks.

Abstract

Infected individuals vary in their contribution to disease persistence, and chronically infected individuals may sustain disease within a population. Pneumonia in wild sheep is one disease that might persist through chronically infected individuals and can precipitate annual lamb mortality as high as 100%. Bacterial *Mycoplasma ovipneumoniae* (*Mo*) presence strongly correlates with lamb pneumonia epizootics, and is a respiratory pathogen of Caprinae commonly present in domestic sheep and goats. Utilizing *Mo*-infected bighorn sheep populations in the Black Hills of South Dakota, we evaluated whether removing chronic shedders of *Mo* improved lamb survival and eradicated juvenile pneumonia mortality. We classified chronic shedders as adults (aged 3+) that tested positive for *Mo* on every nasal swab collected over a 20 month period. Once identified, we removed chronic shedders from our treatment population (Custer State Park) and not in an adjacent control population (Rapid City). We did not detect pneumonia mortality among lambs following treatment, whereas it was the leading source of mortality among control herd lambs. Lamb survival in the treatment population averaged 76% annually, as compared to 35% survival in the control. Overall mortality hazard for lambs up to 0.5 years old was significantly reduced in the treatment population relative to the control ($\beta_{treatment}$=-1.40, CI=-2.42, -0.46). These outcomes support the hypothesis that *Mo* is the primary causative agent of pneumonia epidemics in bighorns and is maintained by chronic shedders in free-ranging populations. Our results of removing chronic shedders and improving lamb survival give wildlife managers options to address low recruitment in pneumonic bighorn sheep populations.

Introduction

Variation among individuals in their contribution to disease spread and persistence has been theorized to serve an important role in sustaining human and wildlife epidemics (Woolhouse et al. 1997, Lloyd-Smith et al. 2005, Paull et al. 2012). One way individuals vary is in their propensity to chronically sustain infections, and research suggests that a few chronically infected individuals can sustain the presence of certain diseases within a population by shedding the responsible pathogen and reinfecting other individuals within their population (Monack et al. 2004, Buhnerkempe et al. 2017). To our knowledge, experimental evidence of disease outbreaks being halted by identifying and isolating such "chronic shedders" remains undocumented in the literature. Chronic shedding may be ubiquitous across a variety of taxa (Wertheim et al. 2005, Buhnerkempe et al. 2017, Plowright et al. 2017). Experimental evidence testing the concept of eliminating chronic shedders to control disease outbreaks would demonstrate the efficacy of this disease management action, and be applicable across the human, domestic animal, and wildlife health sectors.

Recurrent epizootics of pneumonia, believed to be initiated by infection in adults, limit lamb survival and prevent population recovery in bighorn sheep (*Ovis canadensis*) herds across North America (Cassirer and Sinclair 2007, George et al. 2008, Wood et al. 2017). Evidence suggests that domestic Caprinae serve as a reservoir for the responsible infectious agent (Monello et al. 2001, George et al. 2008, Besser et al. 2013, Besser et al. 2017), and that pathogen transmission can occur when bighorns interact with domestic sheep and goats (George et al. 2008,

Besser et al. 2012). Infected bighorns also infect naïve bighorns, causing intraspecies transmission within and between herds (Cassirer et al. 2013). After subsequent transmission within the bighorn population, an all-age epizootic can occur, and may initially kill 40-80% of the herd (Spraker et al. 1984, Enk et al. 2001). The all-age epizootic is often followed by annual juvenile pneumonia epizootics of similar or greater magnitude (Cassirer et al. 2013, Smith et al. 2014). With little annual recruitment, bighorn herds experiencing juvenile pneumonia epizootics exhibit an age structure skewed towards older individuals, which increases the population's vulnerability to extinction (George et al. 2008, Cahn et al. 2011, Manlove et al. 2016). Despite conservation's clear need for methods to improve lamb survival in pneumonic herds, no published study demonstrates a method that clearly achieves this goal.

Historical attempts to curtail pneumonia mortality rates in bighorn sheep populations varied in approach and directed their efforts at a wide variety of causal agents, but met little success. Antibiotics (Coggins and Matthews 1998, Rudolph et al. 2007, McAdoo et al. 2010), vaccination (Cassirer et al. 2001, Sirochman et al. 2012), partial and complete depopulation (Cassirer et al. 1996, McFarlane and Aoude 2010, Bernatowicz et al. 2016), and mineral supplementation (Coggins 2006) have all been tested. One likely reason for the unsuccessful results is that the techniques targeted the wrong etiological agent. Several bacteria (i.e., *Pasteurella multocida*, *Bibersteinia trehalosi, Mannheimia haemolytica*) and parasites (*Protostongylus* spp.) are detected in the lungs of bighorn sheep fatally affected by pneumonia and have been the target of numerous treatments (Foreyt et al. 1994,

Miller et al. 2000, Dassanayake et al. 2009, Grigg et al. 2017)*. Some researchers still

identify leukotoxigenic *Pasteurella* as playing the primary role in pneumonia

development (Dassanayake et al. 2017, Grigg et al. 2017). However, of all potential

candidates, bacterial *Mycoplasma ovipneumoniae* (*Mo*) exhibits the strongest

correlation with bighorn sheep pneumonia mortalities (Besser et al. 2012, Besser et

al. 2013, Cassirer et al. 2018), a trend that was discovered only a decade ago with

access to improved molecular techniques (Besser et al. 2008). Ultimately, the lack of

a clearly successful treatment indicates that more research is needed to develop

management solutions, and that definitively identifying the causative agent of

pneumonia in bighorn sheep would allow wildlife managers and veterinarians to

better direct their limited resources (Cassirer et al. 2018).

Years of studying pneumonic bighorn sheep herds produced useful

epidemiological insights that suggest further avenues of research. One such insight

is that ewe infection is likely responsible for annual juvenile epizootics; most

juvenile pneumonia mortalities occur prior to weaning, and lambs rarely interact

with individuals outside their nursery groups during this timeframe (Cassirer et al.

2013, Manlove et al. 2017). Lambs with both *Mo*-positive and *Mo*-negative dams die

during these outbreaks (Weyand et al. 2018). Secondly, only a small proportion

(median=22%) of bighorns tend to test positive for shedding *Mo* in a given herd at

any point in time, despite high herd seroprevalence (median =67%; Cassirer et al.

2018). Although only based on a single sub-population, a third paper noted that

50% of bighorns remained positive for *Mo* infection after their first positive test,

suggesting chronic carriage in some individuals (Plowright et al. 2017). Based on

this groundwork, it is reasonable to hypothesize that ewes chronically shedding *Mo*
are few in number, responsible for annual pneumonia epizootics in lambs, and
identifiable with adequate testing intensity. A natural prediction of this hypothesis
is that identifying and removing all chronically shedding ewes would reduce or
eliminate juvenile pneumonia mortality and lead to improved survival among
bighorn sheep lambs. Since chronic shedders may maintain disease within a
population in a variety of taxa (Foley et al. 1997, Wertheim et al. 2005,
Buhnerkempe et al. 2017), research designed to test this hypothesis could yield
insights applicable beyond bighorn sheep management.

To test the effect of chronic shedder removal, we utilized free-ranging
bighorn sheep herds near Custer State Park (CSP; treatment herd) and Rapid City
(control herd), South Dakota, USA (Figure 1). Our objective was to document
whether the removal of *Mo* chronic shedders would improve lamb survival.

Methods

The Black Hills are a small, isolated mountain range rising from the Great Plains of
southwestern South Dakota and east-central Wyoming (Froiland 1990) that occupy
an area of approximately 8,400 square kilometers (Fecske et al. 2004). They range
in elevation from 972 meters above sea level to 2,207 meters at Black Elk Peak.
Ponderosa pine (*Pinus ponderosa*) is the dominant tree species (Brown and Sieg
2016). Black Hills spruce (*Picea glauca* Densata) and aspen (*Populus tremuloides*)
increase in abundance at higher elevations in the central and northern Black Hills.
Based on data collected at the Rapid City Airport weather station, average annual
precipitation over the course of our study was 11.3 cm of rainfall and 29.6 cm of

snow. Temperatures ranged from -30°C to 41°C, with an average high of 17°C and an

average low of 1°C (National Oceanic and Atmospheric Administration 2018).

Despite being common in the Black Hills before European settlement, the

introduction of livestock and market hunting led to the local extirpation of bighorn

sheep in 1899 (Seton 1929, Witte and Gallager 2012). Beginning in 1922, managers

and conservationists performed 9 reintroduction events that resulted in current

populations of bighorn sheep in 5 distinct locations: CSP, Rapid City, Elk Mountain,

Hell Canyon, Deadwood (South Dakota Department of Game Fish and Parks 2018).

Only the CSP and Rapid City herds were known to be experiencing pneumonia

epizootics at the time of chronic shedder removal, and researchers verified *Mo*

presence in both (Smith et al. 2015; Table 1). These herds utilized ranges that were

spatially isolated by approximately 12 km straight-line distance (Figure 1). We did

not observe range overlap during the course of the study. Both areas were easily

accessible by U.S. Forest Service and state fire roads. Predator assemblages

consisted of mountain lions (*Puma concolor*), coyotes (*Canis latrans*), bobcats (*Lynx*

rufus), bald eagles (*Haliaeetus leucocephalus*), and golden eagles (*Aquila chrysaetos*)

(Smith et al. 2015).

We designated the CSP herd as the treatment herd, which was located

primarily within a 28,733 ha park in Custer County, South Dakota. Source herds for

the treatment herd were Whiskey Mountain, Wyoming (22 bighorns, 1965) and

Alberta, Canada (20 bighorns, 1999)(South Dakota Department of Game Fish and

Parks 2018). Deep canyons and exposed rocky outcroppings in the central and

northeastern regions of the park characterized bighorn sheep habitat. At the

beginning of the study, 14 ewes, 6 rams, and 2 lambs resided in the treatment

population. No farms inside this study area were known to raise domestic sheep or

goats, but several residents kept domestic sheep within 10 km of the park boundary.

We designated the Rapid City herd as the control herd, which inhabited a

mixture of public and private land in Spring Creek and Rapid Creek canyons near

Rapid City in Pennington County, South Dakota. Control source herds were

Georgetown, Colorado (26 bighorns, 1991) and Badlands National Park, South

Dakota (5 bighorns, 1992) (South Dakota Department of Game Fish and Parks

2018). Bighorn sheep in the control herd generally used canyon bottoms and walls

for parturition and summer range, and then moved to residential lawns closer to

Rapid City for winter range (Smith et al. 2014, Smith et al. 2015). At least one farm

within the control study area kept domestic sheep and goats. Approximately 45

ewes, 20 rams, and 5 lambs populated in the control area at the beginning of the

study.

Adult Collaring, Disease Testing, and PCR Analysis

We chemically immobilized bighorn sheep (1.5-3.0 milliliters BAM; 0.43 mg/kg

butorphanol, 0.29 mg/kg, azaperone, 0.17 mg/kg medetomidine, Wildlife

Pharmaceuticals) via dart rifle (Dan-Inject, Børkop, Denmark, EU) or captured them

by net-gunning from a helicopter (Quicksilver Air, Inc., Fairbanks, AK and Hells

Canyon Helicopters Lewiston, ID). While they were immobilized, we fitted bighorn

sheep with very high frequency (VHF) collars (M252OB; ATS). We also attached

color-coded, numbered tags to the collar and ear of each captured bighorn sheep to

enable individual identification.

Before releasing the bighorns, we collected samples to determine *Mo* carriage and *Mo* antibodies. We collected *Mo* mucosal samples via three nasal swabs, which were consecutively inserted deep into each of the nares and then removed while being slowly rotated around the wall of the nasal cavity (Drew et al. 2014). Two of these swabs were returned to their sheath and one was immersed in a Tryptic Soy Broth media with 15% Glycerol (Hardy Diagnostics; Butler et al. 2017). To detect *Mo* antibodies, we collected blood, from which we obtained serum. We refrigerated all swabs and serum and then shipped them to the Washington Animal Disease Diagnostic Lab (WADDL), or Dr. Thomas Besser's lab at Washington State University (WSU, Pullman, Washington), for analysis.

To detect *Mo* presence and to estimate its abundance in a nasal swab sample, we extracted and amplified bacterial deoxyribonucleic acid (DNA) using realtime polymerase chain reaction (RT-PCR) techniques (McAuliffe et al. 2003, Besser et al. 2008). We deemed a sample to be positive if fluorescence generation exceeded the threshold before the 36th RT-PCR cycle, indeterminate if detected between the 36th and 40th cycle, and negative if undetected through all 40 cycles. Utilizing the collected serum, we determined *Mo* antibody presence by competitive enzyme-linked immunosorbent assay (ELISA) using standard techniques (Ziegler et al. 2014).

Chronic Shedder Identification and Experimental Removal

We commenced *Mo* testing of adult bighorn sheep in the treatment herd in August 2014 and compiled *Mo* histories for each individual in that herd by April 2016. We strove to sample each animal for *Mo* presence 3 times before April but obtained a

minimum of 2 tests on every adult individual before or shortly after experimental manipulation (Table 1). When we compiled all tests for a given individual, we classified them as a chronic shedder (always tested positive after the first positive test), intermittent shedder (negative test after positive tests), or non-shedder (all negative tests). We repeated this process for all individuals in the treatment herd prior to experimental manipulation.

Once we identified chronic shedders in the treatment herd, we relocated them to a penned facility at South Dakota State University (SDSU). Post-removal, we monitored adult bighorn sheep in both the treatment and control herd for *Mo* presence using similar testing techniques.

Lamb Capture

We checked breeding-age ewes for pregnancy via ultrasonography during *Mo* testing in late winter and early spring (2015/16 and 2016/17; E.I. Medical Imaging, Loveland, CO). Following methods described in Smith et al. (2014b) and Bishop et al. (2011), we fitted pregnant ewes with very high frequency (VHF) vaginal implant transmitters (VITs). We monitored VITs daily from the ground using hand-held directional radio telemetry units starting on 15 April each year (Telonics, Inc., Mesa, AZ).

If we detected an expelled VIT, we tracked in on the adult ewe's radio collar to locate her and check for the presence of a lamb. We used latex gloves and minimized handling time to avoid abandonment during radio marking (Smith et al. 2014b). Captured lambs were weighed, fitted with an expandable VHF collar, and

their sex was determined. We recorded GPS waypoints at both the site of the lamb capture and the location of the VIT.

If a VIT malfunctioned, was prematurely ejected, or a ewe was never fitted with a VIT, we performed daily visual checks for lamb presence. We determined age based on its size, ambulatory ability, and associations with other bighorn sheep. If the lamb seemed immobile and the terrain was safely navigable, we attempted hand capture. We used a small netgun to capture older, mobile lambs (ACES, LLC, Broomfield, CO). Lambs that we failed to capture within 2 weeks of birth were immobilized at 2-3 months of age using the ground-based chemical immobilization technique described for adult bighorns but using 0.5 cubic centimeters of BAM. The South Dakota State University Institutional Animal Care and Use Committee approved all capture and handling procedures prior to project initiation (Approval number 16-00A). We developed capture protocol based on recommendations from the American Society of Mammalogists (Sikes and Animal Care and Use Committee of the American Society of Mammalogists 2016).

Lamb Monitoring

To obtain high-quality cause-specific mortality data on bighorn sheep lambs, we needed to investigate and collect cadavers in a timely fashion. Hence, we monitored lambs every day in both study areas using handheld directional antennas, starting on the date of capture and ending on 20 November. The lamb collars were movement-sensitive and changed signal transmission from 40 to 80 pulses/min when the collar was not moved for ≥8 hours. We also attempted to visually observe all lambs when possible because predators and scavengers moved collars as they

consumed the carcass, delaying mortality detection and thereby making a cause-of-death determination difficult.

Upon detecting a mortality signal from the collar, we immediately located the bighorn and assessed the site for evidence indicative of potential causes of death. Specifically, we examined the site for signs of predator presence (scat, tracks, scrapes; Elbroch 2003) and the bighorn carcass for caching, bite marks, hemorrhaging, and skeletal disarticulation (Stonehouse et al. 2016). We also noted if the cadavers were generally still intact, which provided evidence of potential pneumonia or other health-related causes. We shipped fresh cadavers to WADDL for a necropsy and used the test results to supplement field observations in assigning the likelihood of the various causes of death. For all samples, we incorporated our knowledge based on necropsies, evidence at the site, and previous behavioral observations of the individual to assign probabilities to each cause-of-death category (Table 2). Cause-specific mortality categories were "predation", "pneumonia", and "other". This created a vector of probabilities across all categories, and these probabilities summed to one. When cause of death was certain, we created the vector by assigning a single, non-zero entry to the appropriate cause-of-death category.

Survival Analysis

We deemed treatment (i.e., herd identity), year (Gaillard et al. 2000a), individual sex (Rioux-Paquette et al. 2011), birth timing (Feder et al. 2008), and birth weight (Festa-Bianchet et al. 1997) as covariates potentially important in affecting survival of lambs to 0.5 years old. We coded the treatment effect as a constant binary

variable throughout the study, assigning individuals in the treatment herd to "1" and those in the control herd to "0". Similarly, we coded sex as a constant binary variable, giving males a value of "1" and females "0". We assigned birth timing as a categorical variable that consisted of 3 groups: lambs born within ±3 days of the median date of lamb births in a given year (i.e., peak birth group), those born >3 days before the peak period (i.e. early-born group), and those born >3 days after the peak period (late-born group)(Smith et al. 2014a). Birth weight was a continuous variable and measured with a scale to the nearest 0.10 kg. Both birth timing and birth weight could only be accurately measured if the lamb was caught at a time close to parturition; hence, we approximated missing values associated with late-caught lambs through resampling.

After considering the biology and disease ecology of bighorn sheep lambs in the Black Hills, we built *a priori* daily survival models to test relevant hypotheses (Table 3). In our global model (Model 6, Table 3), we calculated log unit cumulative hazard as $\ln(\Lambda_{i,j}) = \gamma + \beta_{treatment} \times treatment_i + \beta_{year} \times year_i + \beta_{sex} \times sex_i + \beta_{birth\ timing}[birth\ timing_i] + \beta_{birth\ weight} \times birth\ weight_i + \rho_j$, where γ was the base-line, log unit cumulative hazard rate. We signified the effect of the treatment as $\beta_{treatment}$, with $treatment_i$ being an indicator for the treatment herd. We denoted the effect of year as β_{year}, with $year_i$ indicating the effect of 2017 on the ith lamb. We assigned β_{sex} to represent the effect of an individual's sex, with sex_i serving as an indicator of males. We denoted the effect of the period of lambing a given lamb was born in as $\beta_{birth\ timing}$, where $\beta_{birth\ timing}[1]$ indicated the effect of the ith individual born in the peak lambing period and $\beta_{birth\ timing}[2]$ indicated the effect of an

individual being born in the late lambing period. We assigned the effect of birth

weight as $\beta_{birth\ weight}$, with $birth\ weight_i$ being the specific birth weight of the ith

individual. We signified the day effect with ρ_j.

Using a novel Bayesian time-to-event survival analysis framework

implemented through Nimble in Program R, we fitted the models to the collected

data (NIMBLE Development Team 2018, Walsh et al. 2018). This framework first

calculated the overall daily hazard of dying irrespective of cause of death (Cross et

al. 2015), using a weakly informative truncated-normal prior on the baseline log

unit cumulative hazard that assumed a mean annual lamb survival of 50% and a

95% probability of lying in the interval ~10% to ~ 80% (specifically,

$\gamma \sim \text{dnorm}[-6.26, \text{precision} = 3]T[-8, -1]$; all priors are specified in the BUGS

language format, Parr et al. 2018). To account for variability and temporal

correlation in the daily hazard rates, we specified an intrinsically conditional

autoregressive prior (ICAR; Heisey 2010, Cressie and Wikle 2011) for the effect of

each day on the overall hazard (ρ_j). Thus, we specified a prior with a uniform

distribution ($\rho_1 \sim dunif(-0.5, \text{precision} = 0.5)$) for the first day effect, and we

specified the effect for the jth day as $\rho_j \sim \text{dnorm}(\rho_{j-1}, \text{precision} = \tau)$. Lastly, we

specified the prior for the precision parameter as: $\tau \sim \text{dgamma}(1, \text{precision} = 1)$

(Heisey 2010). The ICAR prior provided temporal smoothing across daily hazard

estimates. Priors on covariate effects were flat ($\beta_x = \text{dnorm}(0, \text{precision} = 0.01)$).

In the second component of our framework, we calculated cause-specific

mortality by extending Cross et al.'s (2015) methodology to explicitly incorporate

observer uncertainty into parameter estimation (Walsh et al. 2018). Specifically, we

treated the true cause of death for each individual as a latent unknown variable for which we assigned a vector of prior predictive probabilities. These prior predictive probabilities specified the observer's belief that each cause of death of interest was the true cause of death given their assessment of the death site evidence (Table 2). We then imputed the true cause of death using a data augmentation approach that generated a cause of death at each Markov chain Monte Carlo (MCMC) iteration based on a categorical distribution with a parameter vector equal to the prior predictive probability vector specified for that individual. Using random starting values, we ran 3 MCMC chains for 100,000 iterations with the first 10,000 repetitions removed for burn-in. We had 6 missing values for both birth timing and birth weight.

After running our 6 *a priori* models, we calculated Watanabe-Akaike Information Criteria (WAIC) from each model and compared them to identify the models that best described the data (Table 3: Gelman et al. 2014). We considered models that differed by ≤ 2 WAIC as potential alternatives to the selected model with the caveat that we preferred a more parsimonious model (Burnham and Anderson 2002, Arnold 2010). Therefore, we based our conclusions on the parameter estimates from the most parsimonious model with the lowest WAIC value. We calculated 95% credible intervals (CI) for all estimated parameters.

<div align="center">

Results

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Chronic Shedder Testing and Removal

We tested 24 bighorn sheep for *Mo* in the treatment herd prior to finalizing chronic shedder removal: 7 males and 17 females (Table 1). This accounted for all bighorn

sheep known to be present in the treatment study area. We tested each bighorn sheep 1 (n=2), 2 (n=10), 3 (n=10), or 4 (n=2) times for *Mo* presence over 60 samples. Fifty-one (85%) of these samples tested negative, 1 (2%) tested indeterminate, and 8 (13%) tested positive. Two female individuals produced 7 of the 8 positive tests and always tested positive for *Mo*. We classified these bighorns as chronically shedding *Mo* and removed them from the population on 13 March 2016 (Table 1). An ewe that later tested negative on her second sample and died before chronic shedder removal generated the other positive test. One other ewe died before chronic shedder removal, leaving 20 individuals present immediately post-removal. We concluded testing to identify chronic shedders on 8 April 2016.

VIT Deployment, Lamb Capture, and Birth Weights

Between January 2016 and August 2017, we implanted VITs in 59 ewes (31 in 2016, 28 in 2017). 35 of the deployed VITs worked properly (18 in 2016, 17 in 2017). We observed VIT retention rates of 70% (12 retained/17 deployed) in the treatment and 55% (23 retained/42 deployed) in the control. We captured 45 lambs (26 in 2016, 19 in 2017). The mean birth date across study areas was 23 May (median=20 May, SE=1.7 days), with control herd lambs born slightly earlier (mean=22 May, median=19 May, SE=2.5 days) than treatment herd lambs (mean=24 May, median= 26 May, SE=1.7 days).

We calculated the average birth weight of lambs to be 5.05 kg (SE=0.08, *n*=37; 2016=4.99 kg, SE=0.09, *n*=20; 2017=5.1 kg, SE=0.15, *n*=17). Male lambs weighed significantly more than female lambs (males=5.28 kg, SE=0.11, *n*=18; females=4.82 kg, SE=0.11, *n*=19; t=-2.98, df=25 p=0.005).

Mortalities and Recruitment

Twenty-seven (60%) of the captured lambs died within 6 months of 1 May 2016 and 2017. We later excluded two of these individuals from the survival analysis due to attributes that compromised their inclusion in the dataset. Of the 25 lamb mortalities deemed fit for analysis, we observed 7 (5 males, 2 females) in the treatment herd and 18 (6 males, 12 females) in the control herd. These mortalities constituted 35% of all lambs born in the treatment herd and 69% of lambs in the control herd. Using the most likely cause of death based on field and necropsy evidence (see Table 2 for full assigned cause-specific probabilities), we found that 71% of mortalities were due to predation events (n=5) and the remaining 29% were due to other causes (n=2) in the treatment herd (Figure 2[i]). In the control herd, we attributed 39% of mortalities to pneumonia (n=7), 33% to other causes (n=6), 17% to predatory events (n=3), and 11% as equally likely to be several causes (n=2) (Figure 2[i]). An additional 3 lambs died in the control herd in 2016 after the first 6 months (1 pneumonia, 1 human-caused, 1 uncertain). Across studies areas, we noted peak lamb predation on 11 July (median=21 June, SE=17.5 days), peak lamb pneumonia mortality on 8 August (median=3 August, SE=17.4 days), and peak death due to other causes on 6 June (median=4 June, SE=5.8 days) (Figure 3). All confirmed predation events were attributed to mountain lions.

Survival Analysis

We included 43 lambs in the survival analysis. We captured 20 of these lambs as neonates in 2016 (5 in treatment, 15 in control) and another 17 (10 in treatment, 7 in control) in 2017. The remaining 6 lambs were caught several months after birth

in 2016 (4 in treatment herd, 2 in control herd) and left-censored until capture date. Because of their late capture date, we did not gather birth weight or birth date information on those individuals. The earliest lamb birth date was 1 May; therefore we commenced our survival analysis on that date and concluded it 6 months later on 1 November for both 2016 and 2017.

According to WAIC values (w_i =0.99), we found that our data most strongly supported the following model: $ln(\Lambda_{ij}) = \gamma + \beta_{treatment}$ x $treatment_i + \beta_{year}$ x $year_i + \rho_j$]. No other model was within 2 ΔWAIC (Model 1, Table 3). Hence, we calculated and reported the log unit cumulative hazard measurements for each day based on this model (Figure 3). Our selected model suggested that living in a herd where chronic shedders had been removed had a negative mean effect on daily hazard in bighorn lambs ($\beta_{treatment}$=-1.40) that was significant (CI=-2.42, -0.46). This corresponds with an annual lamb survival rate of 76% (CI=24%, 96%) in the treatment herd and 35% (CI=10%, 63%) in the control herd. We observed significantly higher lamb mortality in 2017 than in 2016 (β_{year}=1.15, CI=0.28, 2.02).

We analyzed all mortalities and calculated that the probability of a lamb dying from pneumonia in the treatment herd (probability=6%, CI=0-27%) was significantly lower than in the control herd (probability=48%, CI=24-73%), with a probability difference of -42% (CI=-68%,-12%). Lambs had a 60% probability of dying from predation in the treatment herd (CI=26-87%), which was significantly higher than in the control herd probability of 16% (CI=3-36%; probability difference = 45%, CI 9%-77%). The probability of dying of other causes was similar in both study areas (Figure 2[ii]).

Although other models were not as strongly supported as Model 1, they provided insight on how other effects may have contributed to an individual lamb's hazard. We did not find a significant effect of sex, birth timing, or birth weight (Model 2, β_{sex}=-0.01, CI=-0.84, 0.79; Model 4, $\beta_{birth\ timing}[1] = 0.45$, CI=-0.70, 1.68, $\beta_{birth\ timing}[2] = 0.72$, CI=-0.83, 2.19; Model 5, $\beta_{birthweight}$= -0.07, CI=-0.53, 0.37).

Discussion

Our study is the first to rigorously apply the concept of chronic shedding (Monack et al. 2004, Nouwen et al. 2004, Buhnerkempe et al. 2017) to achieve conservation aims in a free-range setting. It also is the first to present strong evidence that chronic shedder removal results in significantly improved lamb survival. Our results indicate that this technique could be a widely applicable management tool to improve lamb recruitment in ailing bighorn sheep herds, especially given accessible terrain and small populations.

Although we never documented a lamb in the treatment herd actively shedding *Mo* bacteria after chronic shedder removal, a 26 day old lamb from the treatment herd that WADDL necropsied had *Mo* antibodies. This could be interpreted as an indication of lamb exposure to living *Mo* pathogens. However, the seropositive lamb did not exhibit lung damage, and RT-PCR did not detect *Mo* bacteria in samples submitted from the cadaver. Passive transfer from bighorn dams to lambs occurs within 24 hours of birth in ruminants and decays with a half-life of 14-21 days (Weaver et al. 2000, Nowak and Poindron 2006). The process is thought to be similar in bighorn sheep (Highland et al. 2017). The mother of the lamb in question was alive before chronic shedder removal and tested positive for

Mo antibodies, indicating likely exposure to the bacteria. Hence, the antibodies in this lamb were likely acquired from the mother through colostrum.

We expected to see a treatment effect but did not anticipate a significant year effect when designing the study. Given the short temporal time scale and relatively small sample sizes of our study, the significant year term is most likely explained by stochasticity. But it is useful to consider alternative explanations, and we could conjecturally assign it to the increased mountain lion predation we observed on 2017 treatment herd lambs (Figure 2[ii]). Temporal variation in mountain lion predation rates, similar to that reported in our experiment, was recorded in a study of mountain lion consumption of camelid species in Patagonia (Donadio et al. 2010). These authors noted that mountain lion consumption of a given camelid species increased as that species became more abundant. Similarly to those researchers, we also observed this trend of increased consumption with increased abundance, as population size (not including young of the year) in the treatment herd increased from 18 to 26 individuals between the 2016 and 2017 lambing seasons. This is a small increase numerically, but accounts for 44% more bighorns on the landscape in the treatment area in 2017. If mountain lion predation on bighorns continues to increase with growing bighorn population sizes, predator management actions may be warranted to ensure population recovery in the treatment herd (Bourbeau-Lemieux et al. 2011, Rominger 2018).

We included a birth timing effect in our modeling based on its important role in describing bighorn sheep lamb survival in the last study performed in Rapid City (Smith et al. 2014a), but we did not find this effect to be significant. Our lack of a

significant birth timing effect also is contrary to another study performed at Ram Mountain, Alberta, where late birth date corresponded with decreased survival to one year old in male lambs (Feder et al. 2008). One possible explanation for this discrepancy is that we included both male and female lambs in our analysis, whereas Feder et al. (2008) differentiated by sex and noted that males were more likely to be affected by late birth date. It is additionally worth noting that both Feder et al. (2008) and Smith et al. (2014a) performed their survival analyses for the entire year, whereas ours was conducted over the first six months of a lamb's life. Because pneumonia mortality usually occurs in the first six months of life in bighorn sheep lambs (Grigg et al. 2017), our time frame is appropriate for our question of interest. Extending our analysis to include a full year might reveal a stronger effect of birth timing.

In bighorn sheep, Festa-Bianchet et al. (1997) found body weight to have a significant effect on lamb survival, but we did find this effect to be significant. Our lack of a significant weight effect also is contrary to observations of other ungulates (Fairbanks 1993, Cassinello and Alados 1996). Early deaths due to pneumonia in the control herd may have prevented weight from becoming an important determinate of hazard in our experiment. To our knowledge, no published study examined the relationship between birth weight and pneumonia mortality in bighorn sheep herds, but population-level variables such as social group interactions and density dependent factors seem more predictive of dying from pneumonia as a lamb (Monello et al. 2001, Manlove et al. 2014). Also, increased body mass is advantageous for surviving the winter in ungulates (Cook et al. 2004,

Hurley et al. 2014), but we only examined survival through November. Based on our findings, it is likely that birth weight is not an important descriptive variable of hazard until after that lamb escapes the threat of mortality from pneumonia.

Our lamb survival rates in the treatment herd were similar to those observed in studies of other healthy herds of bighorn sheep in South Dakota (Zimmerman 2008, Smith et al. 2014a, Werdel 2017, Parr et al. 2018)(Table 4). Previous studies of healthy herds in the Black Hills and Badlands reported that the percentage of collared lambs surviving ranged between 37.5% and 90% for a given year. Since 90% of our treatment lambs survived in 2016 and 40% survived in 2017, lamb survival in the treatment herd after chronic shedder removal falls within the range of survival rates in other healthy bighorn sheep populations. Similarly, 35% of the collared lambs in our control herd survived in 2016 and 0% survive in 2017, which falls within the range of 0-60% of collared lambs surviving in studies of diseased herds in western South Dakota (Parr et al. 2018, Werdel et al. 2017, Smith et al. 2014a, Zimmerman 2008). This comparison provides further evidence that chronic shedder removal resulted in the treatment herd exhibiting characteristics of a typical healthy population.

Our experiment confirms other findings of improved bighorn lamb survival after *Mo* chronic shedder removal. Weyand et al. (2018) found that lambs comingled with chronic shedder ewes still developed pneumonia, even if their mother was not a carrier. However, lambs born to non-shedders in their study did not contract pneumonia when chronic shedders were removed before lambing. Both sample size and the captive setting limited the extendibility of their results, but based on our

findings, they appear extendable to free-range settings. Similarly, preliminary work in the Hell's Canyon bighorn sheep population compares favorably to our findings. Researchers following the Asotin Creek herd removed 3 chronic shedders and noted 31-54 lambs:100 ewes and no pneumonia in the following lambing seasons, as compared to <10 lambs:100 ewes in the nearby, *Mo*-infected Yakima Canyon herd (Bernatowicz et al. 2016). The results from the Asotin Creek study lacked the experimental design to make definitive statements about causes and effects, but our study improves on this by sampling all individuals in our treatment herd multiple times. Although repeating chronic shedder removal in different locations would improve strength of inference, our study and the evidence gathered in captive settings and Asotin Creek strongly suggest that chronic shedder removal will result in *Mo* clearance in a variety of free-range settings.

<div align="center">**Acknowledgements**</div>

names is for descriptive purposes only and does not imply endorsement by the U.S.

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Table 1: Testing dates and results for bighorn sheep sampled in the treatment herd prior to and shortly after chronic shedder removal, 1 August 2014- 8 April 2016. Individuals who always tested positive after their first positive test were considered chronic shedders, and removed 13 March 2016. Antibody presence indicates exposure to *Mo*.

Treatment Identifier	Sex	PCR Test Result[1]				Chronic Shedder	*Mo* Antibodies[2]	Test Dates
		1	2	3	4			
1	♀	-	-	-	-	No	D	8/1/14, 1/1/15, 10/22/15, 3/1/16
2	♀	+	+	+	+	Yes	D	8/6/14, 4/15/15, 10/22/15, 3/13/16
3	♀	-	-	-		No	D	8/11/14, 11/16/15, 3/13/16
4	♀	-	-	-		No	D	8/26/14, 10/31/14, 9/9/2015
5	♂	-	-	-		No	D	1/15/15, 12/3/15, 3/13/16
6	♂	-	-	in		No	D	1/15/15, 11/5/15, 2/11/16
7	♀	-	-	-		No	D	1/28/2015, 11/4/15, 3/1/16
8	♀	-	-	-		No	D	1/15/15, 9/15/15, 2/29/16
9	♀	-	-	-		No	D	1/15/15, 10/2/15, 3/7/16
10	♀	-	-			No	D	3/20/15, 4/8/16
11	♀	-	-			No	D	3/24/15, 4/8/16
12	♀	-	-	-		No	D	4/6/15, 9/11/15, 3/8/16
13	♂	-	-			No	ND	4/7/15, 4/8/16
14	♂	-	-			No	ND	4/7/15, 3/13/16
15	♂	-	-			No	ND	7/28/15, 3/13/16
16	♂	-	-			No	ND	7/28/15, 3/13/16
17	♀	-	-			No	D	7/30/15, 4/8/16
18	♀	-	-			No	D	9/3/15, 3/13/16
19	♀	+	+	+		Yes	D	10/20/14, 10/31/15, 3/13/16
20	♀	-	-	-		No	D	2/4/15, 4/6/15, 2/22/16
21	♂	-				No	in	4/4/16
22	♀	-				No	ND	4/4/16
23	♀	+	-			No	D	8/26/14, 5/1/15
24	♀	-	-			No	D	3/19/15, 10/20/15

[1] - = Not Detected, + = Detected, in = Indeterminate; [2] D = Detected, ND = Not Detected, in = Indeterminate

Table 2: Cause of death probabilities assigned to all lambs that were included in the survival analysis. For analysis, these probabilities were converted into vectors that summed to one across cause-specific categories for each lamb that died.

Treatment Lamb Identifier	Year	Other	Pneumonia	Predation
1	2016	5%		95%
2	2017			100%
3	2017			100%
4	2017	100%		
5	2017			100%
6	2017	100%		
7	2017			100%

Control Lamb Identifier	Year	Other	Pneumonia	Predation
1	2016	60%	40%	
2	2016	100%		
3	2016		100%	
4	2016	10%		90%
5	2016		100%	
6	2016	100%		
7	2016		100%	
8	2016	100%		
9	2016	100%		
10	2016	34%	33%	33%
11	2016		100%	
12	2017	50%	50%	
13	2017			100%
14	2017	100%		
15	2017		100%	
16	2017		100%	
17	2017		40%	60%
18	2017		100%	

Table 3: Descriptions and rankings of all models tested to explain log unit cumulative hazard $[ln(\Lambda_{ij})]$ for each individual bighorn sheep lamb, 1 May 2016 – 1 November 2016 and 1 May 2017 – 1 November 2017. Ranking is based upon Watanabe-Akaike Information Criteria (WAIC) and is reported with ΔWAIC (difference in WAIC between top model and model being compared) and w_i (WAIC weight). γ is baseline log unit cumulative hazard rate, $\beta_{treatment}$ is the effect of the treatment (whether a lamb was in the treatment or control herd), β_{year} is the effect of year, β_{sex} is the effect of sex, $\beta_{birth\ timing}$ is the effect of birth timing, $\beta_{birth\ weight}$ is the effect of birth weight, and ρ_j is the effect of a given day (j) with a random walk prior for temporal smoothing across estimates.

Model Description	Effects	WAIC	ΔWAIC	w_i
1) Hazard varied by treatment and year	γ, $\beta_{treatment}$, β_{year}, ρ_j	295.7	0	0.995
2) Hazard varied by treatment, year, and sex	γ, $\beta_{treatment}$, β_{year}, β_{sex}, ρ_j	307.0	11.3	0.004
3) Hazard varied by treatment	γ, $\beta_{treatment}$, ρ_j	309.4	13.8	0.003
4) Hazard varied by treatment, year, sex, and birth timing	γ, $\beta_{treatment}$, β_{year}, β_{sex}, $\beta_{birth\ timing}$, ρ_j	441.1	145.5	0.000
5) Hazard varied by treatment, year, sex, and birth timing	γ, $\beta_{treatment}$, β_{year}, β_{sex}, $\beta_{birth\ weight}$, ρ_j	492.4	196.8	0.000
6) Global Model	γ, $\beta_{treatment}$, β_{year}, β_{sex}, $\beta_{birth\ timing}$, $\beta_{birth\ weight}$, ρ_j	640.5	344.8	0.000

Table 4: Comparison of % of lambs surviving and cause-specific mortality sources between bighorn sheep herds in this study and other wild herds recently studied in South Dakota. Percentages of cause-specific mortality contributions are based on most likely cause of death determined from field and necropsy evidence (see Table 2 for full assigned cause-specific probabilities).

Diseased Herds	Collared Lambs Surviving	Pneumonia Contribution	Predation Contribution	Other/Unassignable Contributions
Control Herd	35%, 0%	39%	17%	44%
Smith et al. (2014a)[1]	4%, 8%, 7%	35%	30%	35%
Werdel (2017)[2]	60%	42%	0%	58%
Healthy Herds				
Treatment Herd	90%, 40%	0%	71%	29%
Parr et al (2018)[3]	37.5%	0%	35%	75%
Wieseler (2018)[4]	74%	0%	80%	20%
Zimmerman (2008)[6]	90%, 88%, 88%	NI	NI	NI

[1]Smith, J. B., Jenks, J. A., Grovenburg, T. W., & Klaver, R. W. (2014). Disease and predation: sorting out causes of a bighorn sheep (Ovis canadensis) decline. *PLoS One, 9*(2), e88271

[2]Werdel, T. J. (2017). *Evaluation of the Deadwood Bighorn Sheep Herd Translocation.* (Wildlife and Fisheries Sciences), South Dakota State University, Open PRAIRIE. (1704)

[3]Parr, B. L., Smith, J. B., & Jenks, J. A. (2018). Population Dynamics of a Bighorn Sheep (Ovis canadensis) Herd in the Southern Black Hills of South Dakota and Wyoming. *American Midland Naturalist, 179*, 1-14.

[4]Wiesler, A., Personal Communication, 2018

[5]No information available

[6]Zimmerman, T. J. (2008). *Evaluation of an Augmentation of Rocky Mountain Bighorn Sheep at Badlands National Park, South Dakota.* (Wildlife and Fisheries Sciences), South Dakota State University, Open PRAIRIE. (611)

Figure 1: Ranges of study populations of bighorn sheep in the Black Hills, South Dakota, USA, 2016-2018. The Custer State Park herd is the treatment herd.
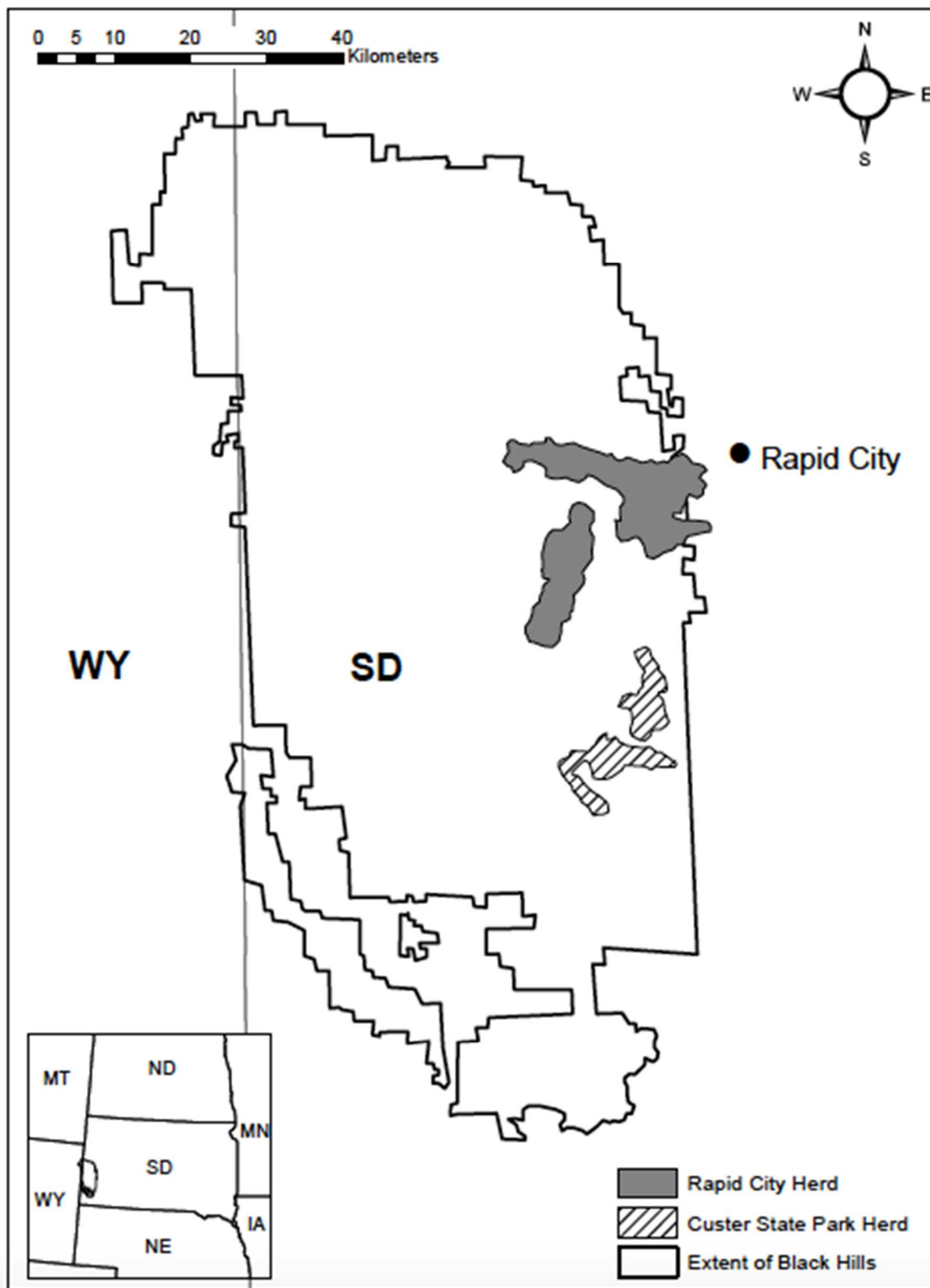
Figure 2: A comparison of mortality sources for lambs between herds after chronic shedder removal in the treatment herd on 13 March 2016. Panel [i] shows cause-specific mortality sources based on the most likely cause of death assigned. No pneumonia was recorded in the treatment herd during the study. Panel [ii] shows the estimated cause-specific mortality probabilities based on the survival analysis. Given that a lamb dies, it is significantly less likely that the mortality will be caused by pneumonia in the treatment herd, but more likely that it will be due to predation. Credible intervals are 95%.
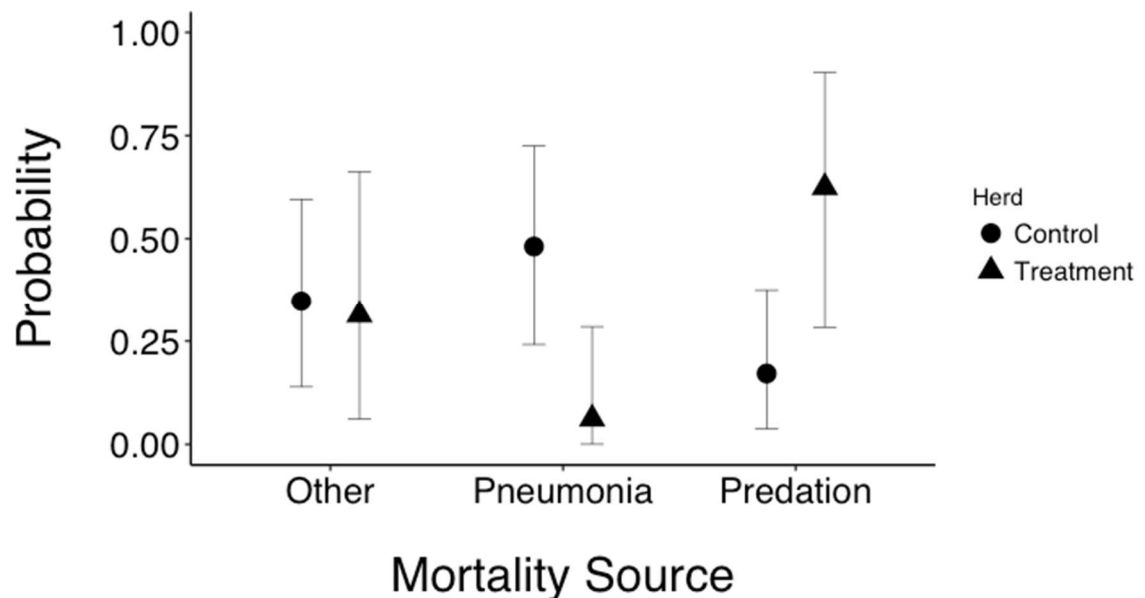
[i]

[ii]
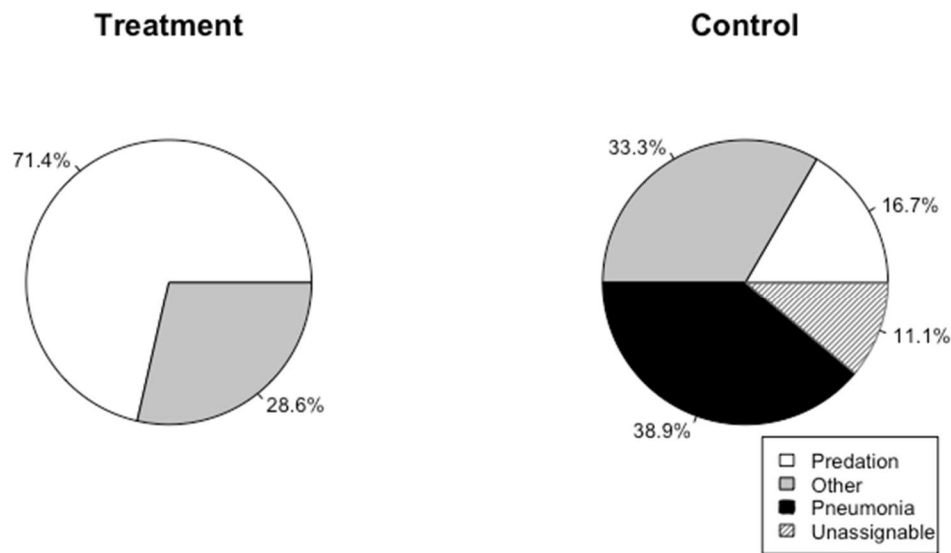
Figure 3: Overall log hazard for a bighorn sheep lamb in Rapid City (γ). Plot is based on our top model, $ln(\Lambda_{ij}) = \gamma + \beta_{treatment} \times treatment_i + \beta_{year} \times year_i + \rho_j$, where $ln(\Lambda_{ij})$ is the unit log cumulative hazard for the ith individual in the jth day, $\beta_{treatment}$ is the effect of being in the treatment herd, $treatment_i$ denotes whether individual i was in the treatment herd, β_{year} is the effect of 2017, $year_i$ denotes whether individual i was born in 2017, and ρ_j is the effect of a given day (j) which is temporally smoothed via a conditional autoregressive random walk prior. Peak periods for each cause specific mortality source are indicated by brackets, and 95% credible intervals are shown in gray. For resolution, X-axis begins on day 7 to remove large CI associated due to low sample sizes early in the year; the first lamb died on day 18.