

Bighorn Sheep and Mountain Goat Transplant Health Assessments



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Project Background:

Respiratory disease is currently having pronounced effects on BHS populations throughout the western US (Aune et al. 1998, Gross et al. 2000, Singer et al. 2000, Cassirer et al. 2007, Cassirer et al. 2013). In Montana, bighorn sheep (BHS) populations have experienced approximately 25 known respiratory disease epizootic events since 1979 (Sells et al. 2015). During the single winter of 2009-10, 4 BHS populations covering a large portion of western Montana experienced simultaneous all-age die off events due to respiratory disease (Edwards et al. 2010).

Translocation is a commonly used tool for the management of BHS in Montana, and has historically been used in mountain goat (MG) population management as well. The need for proactive health monitoring to guide translocation decisions is well known in Montana (Carlsen & Erickson 2010). Research suggests that translocated BHS face higher mortality rates from pneumonia when compared to their resident counterparts (Plowright et al 2013). In addition, there are now several observed cases throughout the western US of pneumonia die-offs in recipient BHS herds following a translocation event, at least raising the possibility that new pathogens or strains were inadvertently introduced or that immunologically naïve transplants fueled local disease transmission. Respiratory disease, and its associated pathogens, serves as one important example of a larger range of infectious organisms that are capable of affecting translocation success in both BHS and MG, and for which proactive monitoring prior to translocation decisions could help to improve the success of these actions. Montana's 2010 Bighorn Sheep Conservation strategy states that Montana will obtain and utilize health profiles for both donor and recipient herds to help guide BHS translocations (Carlsen & Erickson 2010).

Mountain goats and BHS are susceptible to many of the same parasites and pathogens and often overlap in distribution, raising the question of how these two species may affect each other's health, including within the context of translocating one species into another species' range. An outbreak of respiratory disease among sympatric MG and BHS in Nevada in 2013 underscores the potential for these two species to be sharing parasites and pathogens (P. Wolff, Nevada Department of Wildlife, unpublished data).

This report covers the first year of what is intended to be a long-term effort to estimate the pathogen communities and baseline health status of BHS and MG herds across Montana. This information on BHS and MG herds is needed to inform translocation efforts that are intended to help conserve these species, in order to minimize the spread of novel pathogens within and between the two species. We have therefore prioritized pathogen sampling for herds that are likely to be involved in translocation events in the near future, either as donor or recipient herds.

Methods:***Study areas***

Four BHS herds and one MG herd were identified in 2015 by FWP regional wildlife program managers as being of interest for near-future use as translocation source or recipient herds.

These include two potential source herds (one BHS and one MG) and three potential recipient herds (all BHS). All of these herds were sampled for baseline health information (Table 1).

Table 1. Target and actual sample sizes, for winter 2015-2016 sampling of bighorn sheep and mountain goats. Numbers of captured young (<3.5 yrs) and adult (≥3.5 yrs) females, males, and the proportion of lactating adult females are provided. In some cases sample sizes for lactation status are lower than the number of adult females captured because not all adult females were examined for lactation status, and no adult female mountain goats were examined for lactation status.

HD & Herd name	Species	Est. herd size	Recent (5 yr) demographic performance	Target sample size	Information from handled animals				
					Total animals caught	Females <3.5 yrs	Females ≥3.5 yrs	Lactating females ≥3.5 yrs	Males
121: North Clark Fork	BHS	50	Decline	8	3	0	3	1/1	0
122: Clark Fork Cut-off	BHS	30	Decline	8	4	0	4	2/4	0
340: Highlands	BHS	75	Stagnant	16	16	3	12	6/12	1
622: Middle Missouri Breaks	BHS	380	Growth	19	20	8	9	7/9	3
313: Crazy Mountains	MG	350	Stable/decline	20	20*	2	14	NA	3

*one animal that was quickly released due to overheating; no sex determined.

BHS HD 121: North Clark Fork

The North Clark Fork BHS herd is generally located along the Clark Fork River Valley between Thompson Falls and Plains, MT (Figure 1). This herd was founded in 1959 by the reintroduction of 19 sheep from the Gibson Lake North (HD 423) and Wildhorse Island herds. The population reached a high of over 400 sheep in the 1980s, fluctuated around 200 sheep from 1990-2009, and more recently has declined to 50-70 individuals (Figure 2). Mortality due to vehicle collisions is significant within this herd. The current population is comprised of two subgroups. This herd has been suggested as a possible candidate for future herd augmentation.

BHS HD 122: Clark Fork Cut-off

The Clark Fork Cut-off BHS herd is situated between Sesame Creek, Kennedy Creek, and the Clark Fork River (Figure 1). This herd was founded in 1979 and augmented in 1981, in both cases using sheep from Wildhorse Island. The population has generally ranged from 60-140 animals, but following a suspected all-age die-off in 2013, counts have been as low as 18 animals (Figure 2). This herd is currently comprised of just one group of animals. This herd has been suggested as a possible candidate for future herd augmentation.

BHS HD 340: Highlands

The Highland BHS herd is located amidst the Highland and East Pioneer Mountains (Figure 1). This herd was founded in 1967 from the Gibson Lake North (HD 423) herd and augmented seven more times in the following years from six source herds (Castle Reef (HD 422), Bonner (HD 283), East Fork Bitterroot (HD 270), Greenhorn (HD 399G), Ford Creek (HD 424), and Gibson Lake North (HD 423)). The population grew steadily to over 300 sheep until it experienced an all-age die-off in 1995 (Figure 2). The population has failed to recover, ranging between 25-75 animals, with consistently low recruitment rates. This herd has four subgroups with differing apparent health and recruitment rates. This herd has been suggested as a possible candidate for future herd augmentation.

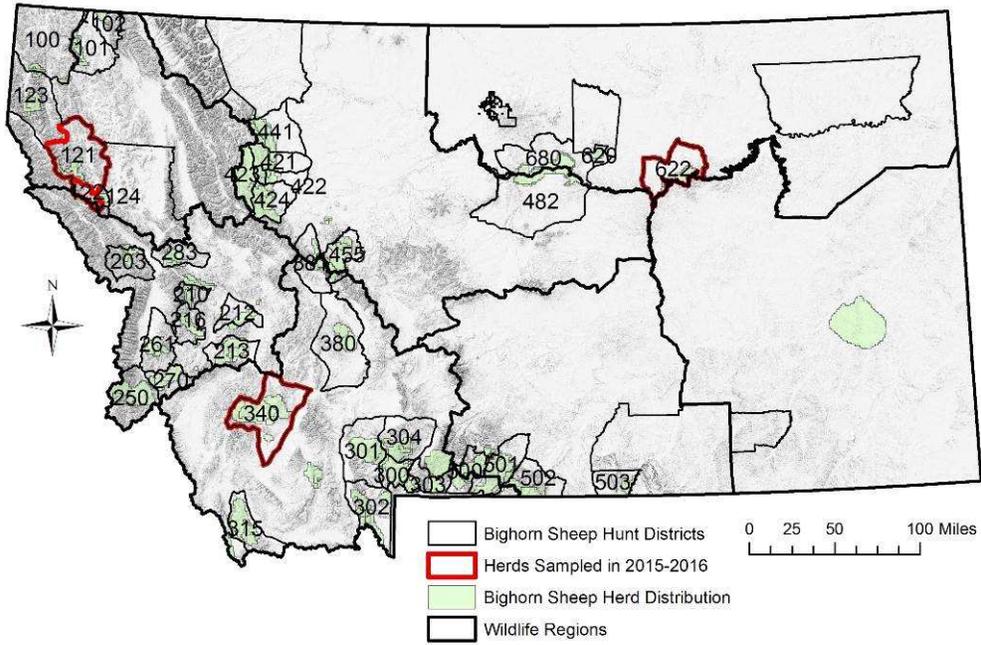
BHS HD 622: Middle Missouri Breaks

The Middle Missouri Breaks BHS herd is located north of Fort Peck Reservoir (Figure 1). This herd was founded in 1980 through a reintroduction effort using BHS from the Gibson Lake North (HD 423) herd. The overall population has steadily increased from the original 28 reintroduced animals to over 300 sheep (Figure 2). This population is comprised of two subgroups. The smaller of the two subpopulations averaged 50 sheep between 1986 and 2010, peaked at 80 sheep in the mid-1990s, and since 2010 has averaged 18 animals with a current low of 10. The larger of the two subpopulations has increased since 2010 from 160 to over 290 sheep. This herd has been suggested as a candidate source herd.

MG HD 313: Crazy Mountains

The Crazy Mountain MG herd is located at the headwaters of Big Timber, Sweet Grass, Porcupine, and Cottonwood Creeks (Figure 1). This herd was founded in 1941 and augmented in 1943 with goats caught in the Deep Creek area west of Choteau (HD 442). The population expanded to over 300 goats by the late 1950s before experiencing an unexplained population crash, after which goat numbers remained low (<100) until the early 1990s (Figure 2). Since the 1990s, the MG population has steadily increased to over 300 goats as of 2013 (Figure 2). This population has as many as eight subgroups, but they tend to congregate and mix on winter range. Thus, for the purposes of sampling, treated MG on the winter range as a single, mixed population. Despite a slight decline in recent years, this herd has historically exhibited long-term population growth, and it has been identified as a potential source herd for future MG translocations.

A



B

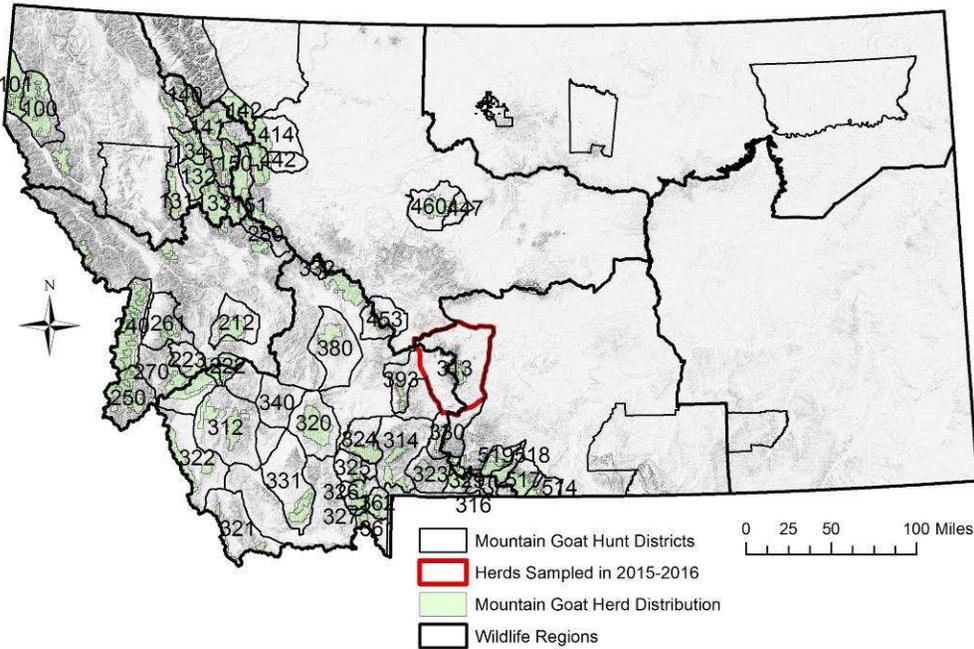


Figure 1. Map of (A) Montana's bighorn sheep and (B) mountain goat hunt districts (outlined and numbered) and associated herd distributions (green polygons). Herds within hunt districts outlined in red were visited for health sampling in 2015-2016.

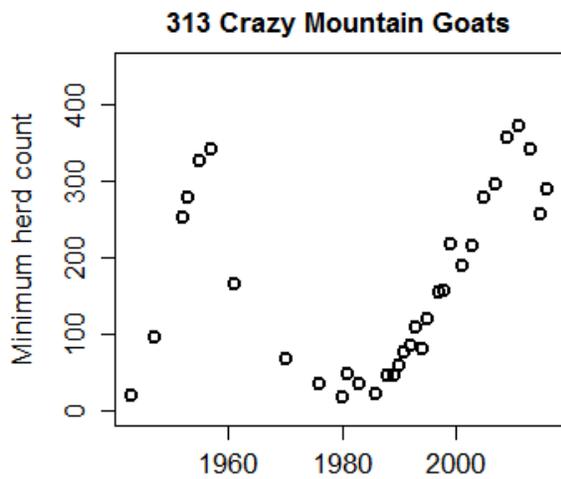
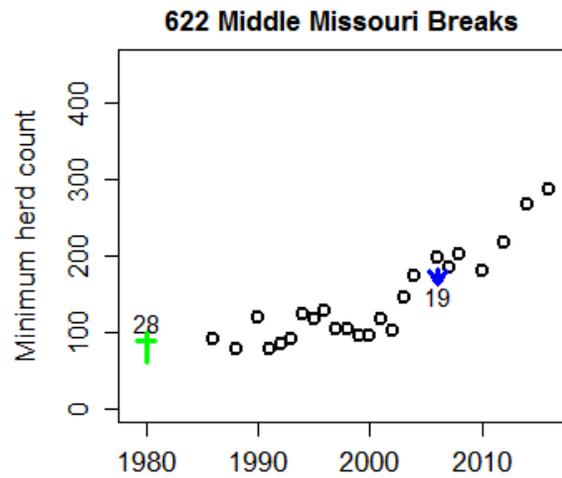
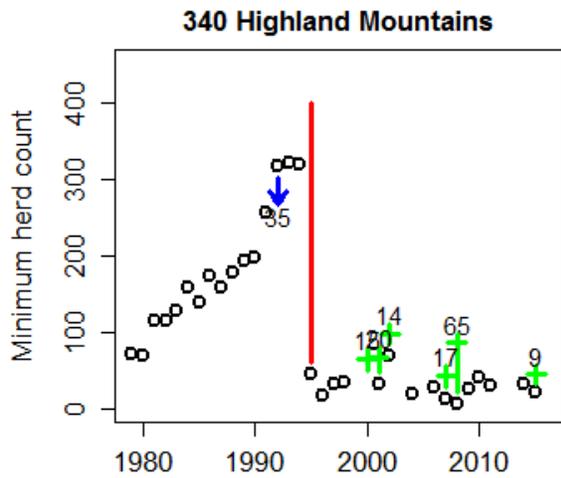
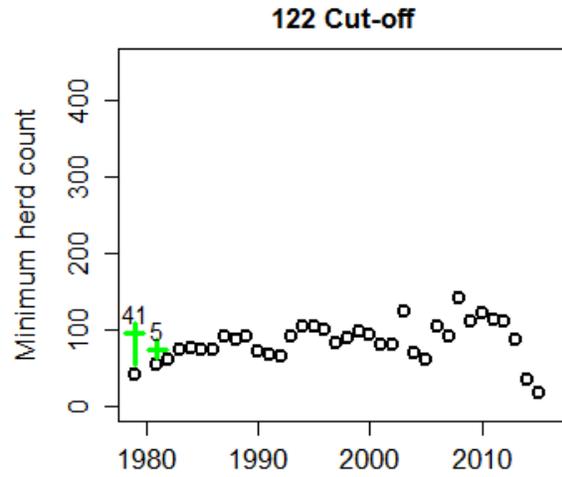
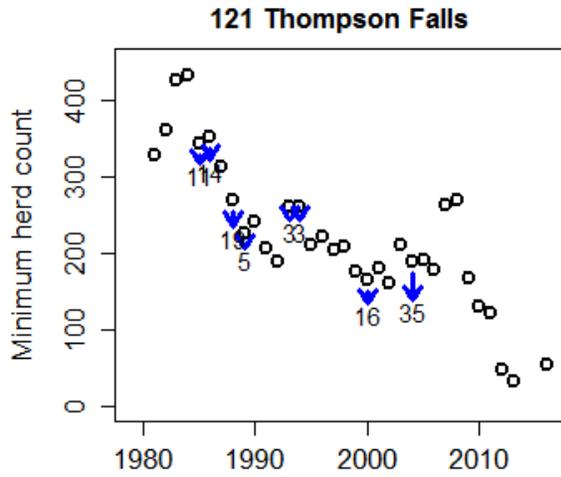


Figure 2. Minimum herd counts over time for the sampled bighorn sheep (HD 121, 122, 340, and 622) and mountain goat (HD 313) herds. Observed pneumonia die-offs are denoted with a red vertical line. Historic translocations into and out of the herds are denoted with green plus and blue arrow symbols, respectively, with the number of animals moved noted. Sheep surveys were conducted December-April; goat surveys were conducted August-September.

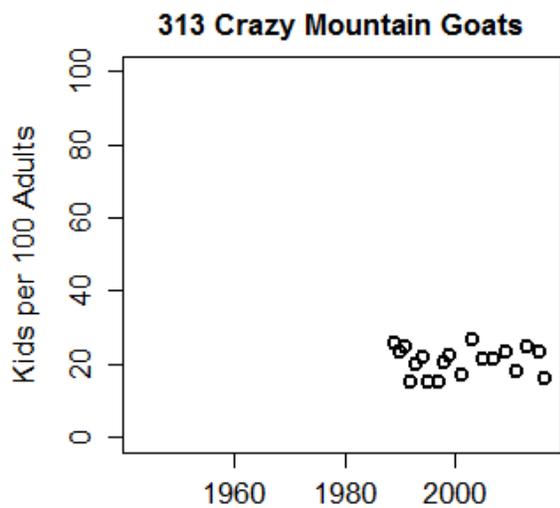
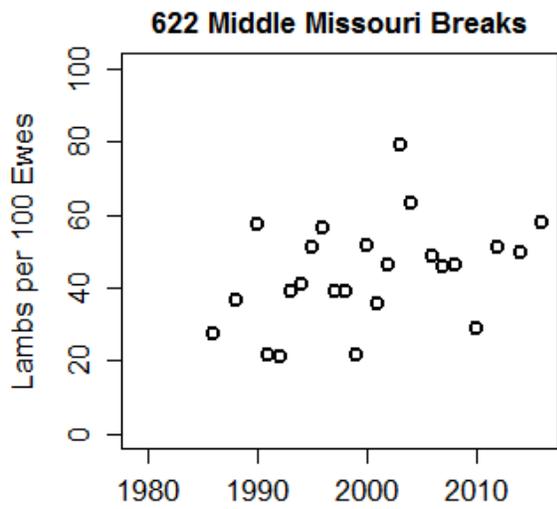
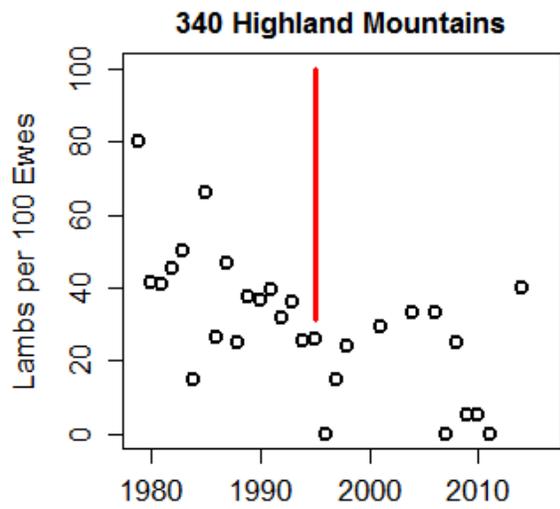
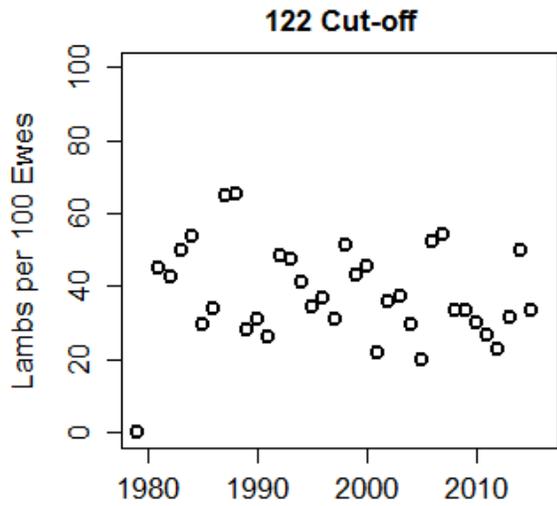
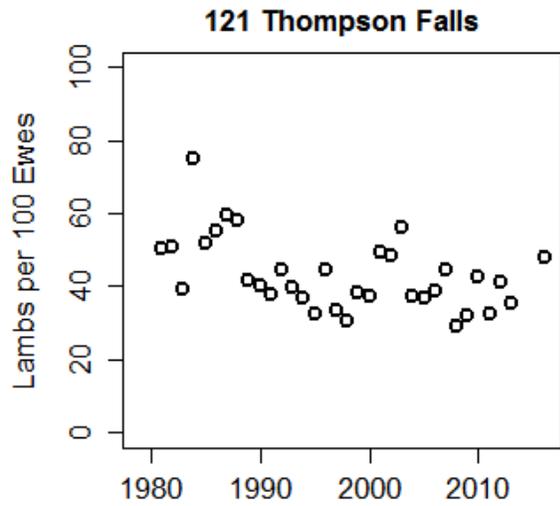


Figure 3. Minimum lambs per 100 ewes, or kids per 100 adults, observed over time for the sampled bighorn sheep (HD 121, 122, 340, and 622) and mountain goat (HD 313) herds. Observed pneumonia die-offs are denoted with a red vertical line. Sheep surveys were conducted December-April; goat surveys were conducted August-September.

Data collection

Sample sizes, animal capture and handling

All capture and handling procedures for sheep and goats were conducted according to the Montana Fish, Wildlife, and Parks Bighorn Sheep Biomedical Protocol (Montana Fish, Wildlife and Parks 2016). Between January and February, 2016, we captured a total of 42 bighorn sheep and 20 mountain goats from the five target populations via ground darting and helicopter net gunning (Table 1). Target sample sizes for each population were defined to detect pathogens with at least 95% confidence, assuming pathogen seroprevalence was between 20-70% at the population level. This seroprevalence range was expected to be sufficient to detect *Mycoplasma ovipneumoniae* (*Movi*) infections based on the available data on seroprevalence rates for *Movi* infections at the time of sampling. Also, at the time these sampling rates were determined, pathogen detection probabilities for our testing methods were not known, and therefore imperfect pathogen detection was not incorporated into our sample size calculations. Based on recent analyses (Butler 2017, Butler et al. *In review*), detection probabilities for many pathogens of interest are lower than previously thought. In future years we will increase sample sizes to detect 10% apparent prevalence and will collect and test duplicate tonsil swabs in order to achieve more reasonable confidence in detecting pathogens in sampled herds, thus accounting for imperfect pathogen detection.

Efforts were made to broadly sample individuals within and across all known subgroups of each herd. We preferentially targeted adult female BHS and MG for capture and sampling. Captured animals were hobbled and blindfolded by the capture crew and either processed on site or transported to a central processing base. Upon restraint, we monitored vital signs while processing each animal. Captured animals were fitted with metal ear-tags prior to release. In addition, VHF radio-collars were deployed on the 16 BHS captured in the Highlands herd to obtain data on survival and spatial structure/movement for local management needs.

Sample Collection

Each captured animal received a full health inspection and evaluation including the collection of information on age, sex, body weight and skeletal length measurements, body condition scores, rump fat thickness (BHS only), lactation status, a genetic sample, whole blood, blood serum, fecal samples, nasal and tonsil swabs (multiple swabs were collected and tested for all animals except the Crazy Mountain goats, where just one swab of each type was collected and tested; swabs were placed in Tryptic soy broth medium and frozen at -80C until tested), and a sampling of any external parasites. A variety of assays were employed to detect (1) a range of parasites and pathogens known to be relevant to BHS and MG health and management (Appendix I; Carlsen & Erikson 2010), (2) trace minerals, and (3) physiological condition (including a body condition score on a scale of 0.5-6 following a protocol developed by Tom Stephenson (personal communication), weight, and maximum rump fat thickness (modified by Tom Stephenson, based on Cook et al. 2010)). Samples were collected and data were analyzed according to standard protocols (Western Association of Fish and Wildlife Agencies 2015). Extra blood serum and swabs were collected and archived for future testing and analyses.

Animal and Field Site Monitoring

Aerial surveys were used to monitor population trend and recruitment ratios in each sampled herd. The area wildlife biologist assigned to each herd conducted aerial surveys (HDs 121 & 122: April with helicopter; HD 340: December-April using helicopter or fixed wing; HD 622: December-February, ground or helicopter; HD 313: August-September using fixed-wing or helicopter). Demographic performance (trend counts and lamb:ewe ratios) may be influenced by herd health. Dramatic change in demographic performance may signal a significant change in herd health, potentially worth evaluating prior to continuing with management plans, particularly involving translocations.

Lab analyses

All BHS and MG pathogen and parasite testing and laboratory use were carried out in accordance with standard protocols from the Western Association of Fish and Wildlife Agencies (Western Association of Fish and Wildlife Agencies 2015; Appendix I). Leukotoxin A PCR testing was conducted on any hemolytic (ability to lyse red blood cells) *Pasteurellaceae* colony, or up to 5 random non-hemolytic colonies. Blood trace mineral levels were analyzed at Michigan State University's Diagnostic Center for Population and Animal Health.

Data analyses

For each herd, we estimated the proportion of the herd exposed (for serology tests) or infected (for PCR or other direct tests) with each pathogen, mean blood trace mineral levels, and mean body condition indices. When estimated detection probabilities for respiratory pathogens were available (Butler 2017, Butler et al. *In review*), we corrected raw estimates of exposure and infection rates by dividing the raw estimates by estimated detection probabilities, and approximated confidence intervals using the delta method. When we failed to detect the presence of a respiratory pathogen, we calculated our statistical power to detect the pathogen if it were present, using the approach detailed in Butler (2017). Statistical power is defined here as the probability (ranging from 0-1) that we would have detected the pathogen if it was present in the herd at 10% prevalence, given the herd size, our sample size, number of swabs collected per animal, and the estimated detection probability. Point estimates and confidence intervals for proportion and probability statistics were estimated with the binomial distribution. Point estimates and confidence intervals for continuous statistics were estimated with the normal distribution.

Results

BHS HD 121: North Clark Fork & BHS HD 122: Clark Fork Cut-off

We encountered difficult capture conditions in HD 121 (n=3) and 122 (n=4) on February 3-4, 2016, so we fell short of our target sample sizes for these herds (Table 1). As a result, we cannot accurately or precisely estimate the prevalence of pathogen infection or exposure in these herds. Our estimated power to detect key respiratory pathogens is too low (all <0.50, most <0.20) to comment on their absence with any certainty (Table 3). However, we did detect several moderate-risk pathogens (Appendix I) in these herds. In HD 121, *Bibersteinia trehalosi*, lungworm, anaplasma, and parainfluenza type-3 (Table 2). In addition, we detected the presence of a leukotoxin positive *Mannheimia* species (e.g. *Mannheimia ruminalis* or other

unidentified *Mannheimia* species). In HD 122, we found evidence of infection with lungworm, anaplasma, and PI3 (Table 2).

Table 2. Summary of raw prevalence (proportion testing positive), 95% confidence intervals (in brackets), and sample sizes for pathogen/parasite exposures by herd. This table does not account for imperfect pathogen detection (see Table 3).

Pathogen	Test Type	Perceived Riskiness	HD 121 BHS	HD 122 BHS	HD 340 BHS	HD 622 BHS	HD 313 Crazy Mountain MG
<i>Mycoplasma ovipneumoniae</i>	PCR	High	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.23, [0.06, 0.54], n=13	0.0, [0.0, 0.21], n=19	0.0, [0.0, 0.20], n=20
<i>Mycoplasma ovipneumoniae</i>	Serology	High	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.63, [0.36, 0.84], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
Leukotoxin A+ <i>Pasteurellas</i>	PCR	High	1, n=1	NA, n=0	1.0, [0.05, 1.0], n=1	NA, n=0	1.0, [0.46, 1.0], n=5
Hemolytic <i>Pasteurellas</i>	Culture	High	0.0, [0, 0.95], n=1	0.0, [0.0, 0.60], n=4	0.06, [0.0, 0.32], n=16	0.0, [0.0, 0.21], n=19	0.0, [0.0, 0.20], n=20
<i>Mannheimia haemolytica</i>	Culture	Moderate	0.0, [0, 0.95], n=1	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.21], n=19	0.0, [0.0, 0.20], n=20
<i>Bibersteinia trehalosi</i>	Culture	Moderate	1.0, [0.05, 1.0], n=1	0.0, [0.0, 0.60], n=4	0.13, [0.02, 0.40], n=16	0.11, [0.02, 0.35], n=19	0.35, [0.16, 0.59], n=20
<i>Pasteurella multocida</i>	Culture	Moderate	0.0, [0, 0.95], n=1	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.21], n=19	0.0, [0.0, 0.20], n=20

Table 2, continued.

Pathogen	Test Type	Perceived Riskiness	HD 121 BHS	HD 122 BHS	HD 340 BHS	HD 622 BHS	HD 313 Crazy Mountain MG
<i>Psoroptes ovis</i>	Clinical	High	Not observed	Not observed	Not observed	Not observed	Not observed
Contagious ecthyma (Orf)	Serology	Moderate	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.25, [0.08, 0.53], n=16	0.05, [0.0, 0.27], n=20	0.0, [0.0, 0.20], n=20
<i>Brucella ovis</i>	Serology	Moderate	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.20], n=20	0.05, [0.0, 0.27], n=20
<i>Anaplasma</i>	Serology	Moderate	1.0, [0.31, 1.0], n=3	1.0, [0.40, 1.0], n=4	0.06, [0.0, 0.32], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
Bovine respiratory syncytial virus (BRSV)	Serology	Moderate	0.0, [0.0, 0.80], n=2	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
Bovine herpesvirus (IBR)	Serology	Moderate	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
Parainfluenza-3 virus (PI3)	Serology	Moderate	1.0, [0.31, 1.0], n=3	1.0, [0.40, 1.0], n=4	0.75, [0.47, 0.92], n=16	0.9, [0.67, 0.98], n=20	0.75, [0.51, 0.90], n=20
Ovine progressive pneumonia (OPP)	Serology	Moderate	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.0, [0.0, 0.24], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
Bovine viral diarrhea II	Serology	Moderate	0.0, [0, 0.69], n=3	0.0, [0.0, 0.60], n=4	0.06, [0.0, 0.32], n=16	0.0, [0.0, 0.20], n=20	0.0, [0.0, 0.20], n=20
<i>Leptospira</i> (CAN, ICT, HAR, GRIP, POM serovars)	Serology	Low	0.67, [0.13, 0.98], n=3	0.25, [0.01, 0.78], n=4	0.38, [0.16, 0.64], n=16	0.2, [0.07, 0.44], n=20	0.05, [0.0, 0.27], n=20
Epizootic hemorrhagic disease (EHD)	Serology	Low	0.0, [0, 0.95], n=1	0.0, [0.0, 0.60], n=4	0.06, [0.0, 0.32], n=16	0.2, [0.07, 0.44], n=20	0.0, [0.0, 0.20], n=20
Bluetongue virus (BTV)	Serology	Low	0.0, [0.0, 0.80], n=2	NA, n=0	NA, n=0	NA, n=0	0.0, [0.0, 0.20], n=20
Lungworm	Baermann Fecal Float	Moderate	1.0, [0.05, 1.0], n=1	1.0, [0.40, 1.0], n=4	1.0, [0.56, 1.0], n=7	1.0, [0.60, 1.0], n=8	0.7, [0.35, 0.92], n=10
Coccidia	Fecal Floatation	Low	1.0, [0.05, 1.0], n=1, moderate	0.5, [0.15, 0.85], n=4, few	0.29, [0.05, 0.70], n=7, few- moderate	0.38, [0.10, 0.74], n=8, moderate	1.0, [0.66, 1.0], n=10, moderate- many
Other parasites	Fecal Floatation	Low	1.0, [0.05, 1.0], n=1	1.0, [0.40, 1.0], n=4	1.0, [0.56, 1.0], n=7	0.63, [0.26, 0.90], n=8	0.9, [0.54, 0.99], n=10

Table 3. Summary of estimated raw (apparent) and adjusted (true) prevalence of select respiratory pathogens, accounting for imperfect detection probabilities (Butler 2017). Adjusted prevalences were calculated by dividing the raw estimates by estimated detection probabilities, and corrected 95% confidence intervals (in parentheses) were calculated using the delta method. We estimated the statistical power to detect the pathogen if it were present at 10% prevalence given the herd size, our sample size, number of swabs collected per animal, and the detection probability using the approach detailed in Butler 2017. Point estimates and 95% confidence intervals (in parentheses) for raw prevalences were estimated using the binomial distribution.

Herd	Herd size Sample size Swabs/ animal	<i>Mannheimia haemolytica/glucoSIDA</i> (hemolytic/LeukA+)			<i>Mannheimia spp.</i> (hemolytic/LeukA+)			<i>Bibersteinia trehalosi</i> (hemolytic/LeukA+)			<i>Pasteurella multocida</i>			<i>Mycoplasma ovipneumoniae</i> (PCR)		
		Raw Prev	Adj. Prev	Power	Raw Prev	Adj. Prev	Power	Raw Prev	Adj. Prev	Power	Raw Prev	Adj. Prev	Power	Raw Prev	Adj. Prev	Power
HD 121 BHS	50 1 4	0, (0, 0.98)	-	0.13	1, (0.03, 1)	1	0.07	0, (0, 0.98)	-	0.17	0, (0, 0.98)	-	0.07	0, (0, 0.98)	-	0.26
HD 122 BHS	30 4 2	0, (0, 0.6)	-	0.18	0, (0, 0.6)	-	0.09	0, (0, 0.6)	-	0.22	0, (0, 0.6)	-	0.1	0, (0, 0.6)	-	0.34
HD 340 BHS	75 16 2	0.06, (0, 0.3)	0.13, (0, 0.65)	0.53	0, (0, 0.21)	-	0.3	0, (0, 0.21)	-	0.62	0, (0, 0.21)	-	0.32	0.23, (0.06, 0.54)*	0.25, (0, 0.51)*	0.8
HD 622 BHS	380 19 2	0, (0, 0.18)	-	0.6	0, (0, 0.18)	-	0.36	0, (0, 0.18)	-	0.69	0, (0, 0.18)	-	0.38	0, (0, 0.18)	-	0.85
HD 313 Crazy Mtn. MG	350 20 1	0.25, (0.09, 0.49)	0.92, (0,1)	0.42	0, (0, 0.17)	-	0.22	0, (0, 0.17)	-	0.52	0, (0, 0.17)	-	0.23	0, (0, 0.17)	-	0.78

*Three of the 16 samples were indeterminate, thus prevalence is calculated assuming 13 animals were sampled.

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for sheep in HD 121 & 122 (Table 4) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Serum-based selenium concentrations in both hunt districts fell below the published reference ranges for wild and domestic sheep, although small sample sizes make it difficult to determine whether this reflects a herd-wide pattern.

Table 4. Mean trace mineral concentrations in serum for cobalt, copper, iron, manganese, molybdenum, selenium, and zinc (displayed in parts per million, ppm), along sample sizes and 95% confidence intervals (in brackets) for each sampled herd.

HD	Sample size (n)	Cobalt (ppm)	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Molybdenum (ppm)	Selenium (ppm)	Zinc (ppm)
121	1	0.0007, [NA]	0.86, [NA]	1.3, [NA]	0.0024, [NA]	0.0072, [NA]	0.03, [NA]	0.51, [NA]
122	4	0.0008, [0.0005, 0.001]	0.75, [0.66, 0.83]	1.37, [1.04, 1.7]	0.003, [0.0017, 0.0042]	0.0124, [0.0085, 0.0163]	0.04, [0.03, 0.04]	0.7, [0.59, 0.8]
340	10	0.0007, [0.0005, 0.0009]	0.89, [0.73, 1.04]	1.56, [1.3, 1.81]	0.0037, [0.0029, 0.0045]	0.025, [0.0124, 0.0377]	0.07, [0.05, 0.09]	0.72, [0.62, 0.81]
622	8	0.0013, [0.0009, 0.0016]	0.69, [0.47, 0.9]	1.79, [1.46, 2.12]	0.0028, [0.0024, 0.0031]	0.0237, [0.0022, 0.0451]	0.13, [0.12, 0.15]	0.84, [0.63, 1.04]
313	8	0.0011, [0.0008, 0.0014]	0.65, [0.52, 0.78]	1.4, [1.15, 1.65]	0.0036, [0.001, 0.0062]	0.0034, [0.0, 0.009]	0.06, [0.05, 0.07]	0.93, [0.81, 1.05]

Limited body condition indices, as captured by ultrasound-measured maximum rump fat and body condition score by palpation, are summarized for HD 122 in Table 5. Currently, there is no published literature relating measurements of rump fat to estimates of ingesta-free body fat and pregnancy rates in wild sheep, although such work is forthcoming (Tom Stephenson, personal communication).

Table 5. Body condition indices summarized for each sheep herd, broken down by lactation status among adult (≥ 3.5 yrs) ewes. Mean ultrasound-measured maximum rump fat (cm) and body condition scores (scale of 0.5-6, following a protocol developed by Tom Stephenson, personal communication) assessed by palpation are displayed along with 95% confidence intervals within brackets. Sample sizes are noted.

HD	Date sampled	Mean lactating maximum rump fat (cm)	Mean non-lactating maximum rump fat (cm)	Mean lactating body condition score	Mean non-lactating body condition score
121	2/3/2016	NA	NA	NA	NA
122	2/4/2016	0.35, [0.29, 0.41], n=2	0.33, [0.19, 0.47], n=2	3.5, [3.5, 3.5], n=2	3.3, [2.3, 4.2], n=2
340	2/4/2016	0.32, [0.23, 0.40], n=6	0.485, [0.38, 0.59], n=6	2.8, [2.6, 3.1], n=6	3.6, [3.1, 4.1], n=6
622	1/26/2016	0.26, [0.14, 0.37], n=7	0.65, [0.07, 1.22], n=2	2.7, [2.4, 3.1], n=7	4.0, [2.5, 5.5], n=2

BHS HD 340: Highlands

The 16 sheep captured in HD 340 on February 4, 2016 spanned 4 sub-herd social groups identified by the regional biologist using radio-telemetry data. At the herd level, after accounting for detection probabilities, an estimated 25% (95% CI: 0-51%) had active *Movi* infections (by PCR), and 63% (95% CI: 36-84%) exhibited serological evidence of past or recent infection with *Movi* (Table 2). We also detected the presence of other moderate to high-risk pathogens (Appendix I), including: haemolytic *Pasteurellaceae*, Leukotoxin A+ *Pasteurellaceae*, *Bibersteinia trehalosi*, lungworm, contagious ecthyma, anaplasma, parainfluenza type-3, and bovine viral diarrhea I & II. Some of the sheep captured exhibited significant mucus running from their noses, a possible sign of active respiratory infection.

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, selenium, and zinc for sheep in HD 340 (Table 4) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011).

Body condition indices, as captured by ultrasound-measured maximum rump fat and body condition score by palpation, indicated that lactating adult (≥ 3.5 yrs) females had lower fat reserves than their non-lactating adult counterparts (Table 5). Overall, body condition indices for lactating and non-lactating adult ewes in HD 340 ranked in between those measured in HD 122 and 622.

BHS HD 622: Middle Missouri Breaks

Among the 20 sheep captured in HD 622 on January 26, 2016 (Table 1), we detected several moderate-risk pathogens (Appendix I), including: *Bibersteinia trehalosi*, lungworm, contagious ecthyma, parainfluenza type-3 (Table 2). We did not detect *Movi* by PCR or serology. Given herd size, our sample size, and duplicate swabs per animal, we estimate that we had an 85% probability of detecting *Movi* by PCR if it were present at $\geq 10\%$ prevalence within this herd.

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, selenium, and zinc for sheep in HD 622 (Table 4) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011).

Similar to HD 340, body condition indices were lower among lactating adult ewes than their non-lactating adult counterparts (Table 5). Non-lactating ewes in HD 622 had the highest measures of rump fat and body condition scores of the three measured populations, whereas lactating ewes in this herd had among the lowest body condition indices.

MG HD 313: Crazy Mountains

Among the 20 mountain goats sampled in HD 313 on February 24, 2016 (Table 1), we detected moderate to high-risk pathogens (Appendix I), including: leukotoxin A+ *Pasteurellaceae*, *Bibersteinia trehalosi*, lungworm, *Brucella ovis*, parainfluenza type-3, and bovine viral diarrhea (Table 2). We did not detect *Movi* by PCR or serology, nor were any hemolytic *Pasteurellaceae* detected. Given herd size, our sample size, and single swabs collected per animal, we estimate that we had a 78% probability of detecting *Movi* by PCR if it were present at $\geq 10\%$ prevalence within this herd.

We are unaware of published trace mineral reference values for mountain goats. However, the concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for goats in HD 313 (Table 4) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Selenium levels were lower than reference values for sheep, but within ranges previously reported for mountain goats (Samson et al. 1989).

We did not measure ultrasound rump fat on mountain goats in HD 313, as the procedure has not been validated for goats. We did estimate a body condition score, although this was collected without accompanying data on lactation status. Mean body condition score for adult (≥ 3.5 yrs) nannies was 3.8 (95% CI: 3.5-4.1). We were unable to find any published reference values for body condition in mountain goats.

Discussion

In 2015, we conducted health assessments on four bighorn sheep herds and one mountain goat herd to help inform decisions regarding their use as potential source (BHS HD 622 and MG HD 313) or recipient herds (BHS HDs 121, 122, and 340) for future translocations. Small sample sizes due to difficult capture conditions in HDs 121 and 122 limit our ability to adequately assess the pathogens present in these herds; they will be revisited again in 2016-2017 to boost sample sizes. All other herds exhibited some evidence of infection with several pathogens of concern should they be mixed with naïve sheep/goats (Table 2). In particular, the highest diversity of high-risk agents involved in respiratory disease were detected in HD 340 (*Movi*, and Leukotoxin A+ and hemolytic *Pasteurellaceae*) and among the mountain goats in HD 313 (Leukotoxin A+ *Pasteurellaceae*, although no corresponding hemolysis was detected). However,

we must caution that in most cases where we failed to detect risky respiratory pathogens, our statistical power to detect these pathogens was often too low (generally <0.20; Table 3) to declare their absence from the herd with any confidence.

Trace element concentrations in each sampled herd appeared adequate according to previously published reference ranges in wild and domestic sheep and goats, with the exception of selenium, for which concentrations were low in HDs 121 and 122 (although small sample sizes limit the reliability of these estimates). Body condition among adult female bighorn sheep was associated with lactation status, as has been noted in other species elsewhere (Cook et al. 2013, Proffitt et al. 2016). Across adequately sampled populations, body condition was highest among non-lactators in HD 622, followed by 340, whereas the opposite relationship was observed among lactating ewes (body condition highest in 340, followed by 622). Pregnancy results were not available at the time of this report, so it is possible that pregnancy status further contributed to these body condition patterns. Currently, there is no published literature relating measurements of rump fat to estimates of ingesta-free body fat and pregnancy rates in wild sheep, although such work is forthcoming (Tom Stephenson, personal communication).

Respiratory disease has been identified as one of the largest and most damaging health issues facing BHS (and in some cases MG). Although debate over the most important pathogens involved in BHS respiratory disease continues, Besser et al. (2012), Besser et al. (2013), and Cassirer et al. (*In review*) point to *Movi* as a necessary agent involved in pneumonia outbreaks. However, it is worth noting that among 14 herds in Montana (n=7) and in Wyoming (n=7) that have tested positive for *Movi* in recent years, 29% have never experienced a documented pneumonia epidemic, 79% exhibited adequate lamb recruitment (lamb to ewe ratios >0.20), and at least 57% of the herds met population objectives and/or had stable or positive estimated growth rates (Butler 2017). Conversely, we are unaware of populations that have a recent or more distant history of pneumonia epizootics in which *Movi* has not been detected, since diagnostic tools for *Movi* have become available. Therefore, based on the evidence accumulated to date, *Movi* may be a necessary agent involved in pneumonia epizootics, but may not be a sufficient indicator of a history or high likelihood of pneumonia epizootics. We hope that with future sampling across additional herds, we will have more information with which to evaluate the role of *Movi* and other co-infecting agents in wild sheep and goat respiratory disease.

Our recent detection of *Movi* in HD 340 supports results from previous laboratory submissions from this herd. In addition to *Movi*, this herd also tested positive for the Leukotoxin A gene and hemolytic *Pasteurellaceae*, and several animals handled on capture exhibited mucus running from the nose, a clinical sign of an active upper respiratory infection. These observations of pneumonia symptoms and chronic low lamb recruitment within this herd, combined with detection of multiple risky pathogens, are in line with this herd having a chronic, ongoing pneumonia issue. Given our sampling effort among sheep in HD 622 and among goats in HD 313, our estimated power of detecting *Movi* by PCR, should it be present at 10% prevalence, was 0.85 and 0.78, respectively. These two herds also tested negative for *Movi* by serology; serological tests typically indicate past exposure and are generally recognized as the more

sensitive test to assess recent or current infection at the herd level (i.e., an animal doesn't have to be actively shedding the pathogen to be detected as being or having been infected). These two pieces of information combined suggests that *Movi* may be absent from HD 622 and among the goats in HD 313.

Pasteurellaceae, particularly hemolytic strains or those that contain (and express) the Leukotoxin A gene, are thought to play important roles in respiratory disease, influencing patterns of morbidity and mortality (Dassanayake et al. 2010, Besser et al. 2012, Shanthalingam et al. 2014, Wood et al. 2016). Recent work has indicated that detection probabilities are extremely low (0.12-0.36) for the majority of *Pasteurellaceae* using culture-based diagnostic methods (Butler 2017, Butler et al. *In review*, Walsh et al. 2012). Our sampling effort was designed prior to having this information in hand. Given our low statistical power to detect these pathogens (Table 3), it is likely that we failed to detect *Mannheimia haemolytica*, *Pasteurella multocida*, or additional hemolytic or Leukotoxin A+ *Pasteurellaceae* in some of these herds. HD 622 will be re-sampled in 2016-2017, so additional information on this herd will be forthcoming. Despite these low detection probabilities, we did detect the Leukotoxin A gene in bighorn sheep in HD121 and HD340, and among the goats of HD313, and hemolytic *Pasteurellaceae* in bighorn sheep in HD340. Future work will include both larger sample sizes and repeated samples from the same individual to increase our probability of detecting these *Pasteurellaceae*.

Management Implications

Given that the bighorn sheep of HD 340 are actively infected with high-risk respiratory pathogens, we would recommend against augmenting this herd with additional animals. This herd has experienced at least six augmentations since it crashed during a pneumonia epizootic in 1994, none of which have resulted in significant population growth. Susceptible sheep placed into a herd infected with respiratory disease are not only likely to experience elevated mortality rates due to subsequent infection (Plowright et al. 2013), but are also likely to prolong the natural or spontaneous recovery of the herd by delaying pathogen extinction (Almberg et al. *In preparation*). Alternatively, this herd could be a potential candidate for other, experimental management approaches, such as a test and remove experiment, where *Movi* PCR positive ewes are selectively removed. Several such experiments are underway in other states. Small herd size, existing knowledge of herd substructure, and infections that appear limited to only some sub-groups might facilitate such an approach.

Due to challenging capture conditions, we only obtained small sample sizes in HD 121 & HD 122, which limits our ability to comment on the status of the pathogen community in these herds with any confidence. We are scheduled to revisit these herds in 2016-2017 to increase sample sizes.

We did not detect any of the high-risk respiratory pathogens in samples collected from the bighorn sheep in HD 622, but as stated earlier, our power to detect the leukotoxin positive/hemolytic *Pasteurellaceae* pathogens was insufficient to declare their absence (Table 3). We did detect evidence of exposure to moderately risky pathogens including *Biberststeinia*

trehalosi, lungworm, contagious ecthyma, parainfluenza type-3, but with the exception of contagious ecthyma (for which we found only one positive), most of these pathogens appear to be common across herds without apparent or easily detectable adverse effects. Our confidence in this assessment will be improved by the scheduled research capture and sampling effort in 2016-2017 for a different project (Federal Aid in Wildlife Restoration Grant W-159-R). Updated estimates of pathogen presence/prevalence and uncertainty will be provided to managers to assist with making decisions regarding the suitability of this herd as a source population.

We detected Leukotoxin positive *Mannheimia glucosida* among the mountain goats in HD 313. Although we did not detect any of the other high-risk hemolytic/leukotoxin positive *Pasteurellaceae*, our power to detect these organisms was insufficient to declare their absence (Table 3). Interestingly, the detection of the leukotoxin gene in this herd was not associated with hemolytic activity of the bacteria (the ability of the bacteria to lyse red blood cells), which is assumed to be the sign of toxin expression (Fisher et al. 1999). This indicates that the gene is present in the *Pasteurellaceae* bacteria we found, but that perhaps it was not being expressed; it is unknown how this relates to future risk of gene expression and associated hemolytic activity. We also detected exposure to moderately-risky pathogens including *Biberststeinia trehalosi*, *Brucella ovis*, lungworm, parainfluenza type-3, and bovine viral diarrhea I in this herd. Many sheep herds commonly host these pathogens without apparent adverse effects, so we are unsure about the risk these detections represent. We did not detect infection by *Movi* on PCR (estimated power of detection given 10% prevalence was 0.78) or by serology. The suitability of this herd as a source for augmentation or reintroduction depends on one's comfort with the uncertainty around the limited possibility of the presence of *Movi* (without detection) and substantial uncertainty about the presence of *Pasteurellaceae*, in particular other leukotoxin positive/hemolytic *Pasteurellaceae*.

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Appendix I

All captured mountain goats and bighorn sheep will be screened for the following list of pathogens and parasites. This table also includes an assessment of the risk associated with each pathogen/parasite and an associated rationale, as well as the test types and laboratories employed for the screening. Testing laboratory abbreviations: WADDL=Washington Animal Disease Diagnostic Laboratory; FWP =Montana Fish, Wildlife, and Parks Health Laboratory; DOL=Montana Department of Livestock Laboratory; MSU DCPAH=Michigan State University Diagnostic Center for Population and Animal Health.

Organism	Risk category	Rationale	Test type and any limitations	Testing Laboratory
<i>Mycoplasma ovipneumoniae</i>	High	Likely necessary, if not sufficient, for chronic or epidemic BHS pneumonia.	Serology—gives us most conservative metric of presence/absence from a herd. PCR—the proportion of actively infected individuals may be small and chronically infected individuals may shed intermittently, so a “negative” at the herd level may not give us as much confidence.	WADDL
Leukotoxin A+ <i>Pasteurellas</i>	High	Identified as a potentially important class of pathogens involved in respiratory disease.	PCR	WADDL
Hemolytic <i>Pasteurellas</i>	High	Identified as a potentially important class of pathogens involved in respiratory disease.	Culture	WADDL
<i>Mannheimia haemolytica</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Bibersteinia trehalosi</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Pasteurella multocida</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Psoroptes ovis</i>	High	Highly contagious and capable of causing chronic and extensive morbidity and mortality.	Parasitology	FWP/DOL
Lungworm	Low	Depending on burden; low burdens are normal, high burdens may be problematic.	Parasitology	MSU DCPAH
Contagious ecthyma (Orf)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
<i>Brucella abortus</i>	Low	Not generally considered a problem for sheep/goats.	Serology	DOL

<i>Brucella ovis</i>	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
<i>Anaplasma</i>	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine respiratory syncytial virus (BRSV)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine herpesvirus (IBR)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Parainfluenza-3 virus (PI3)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Ovine progressive pneumonia (OPP)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine viral diarrhea (BVD I & II)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bluetongue virus (BTV)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Epizootic hemorrhagic disease (EHD)	Low		Serology	DOL
<i>Leptospira</i> (CAN, ICT, HAR, GRIP, POM serovars)	Low	Environmentally transmitted and widely distributed. Probably not a major concern.	Serology	DOL