

# Bighorn Sheep and Mountain Goat Herd Health Assessments



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**STATE:** *Montana*  
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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 understood 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 could improve the success of these actions. Montana Fish, Wildlife, and Parks' (FWP) 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 the translocation of 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 second year of what is intended to be a long-term effort to evaluate 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, and to minimize the introduction and spread of novel pathogens within and between the two species. As part of FWP's Bighorn Sheep and Mountain Goat Health Program, we have developed a sampling and monitoring plan consistent with programs in other western states and which prioritizes 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 were identified in 2016 by FWP regional wildlife program managers as being of interest, either to inform current management or for near-future use as translocation source or recipient herds. These included two potential source herds (HD 102, 680) and two potential recipient herds (HDs 101, 122). In addition, 65 BHS were translocated out of HD 482 to reduce population density. These animals were released into Sheep Creek and the Beartooth Wildlife Management Area in HD 455. We attempted to sample these herds for baseline health information (Table 1), however, weather, low densities of sheep, and difficult capture terrain precluded adequate sampling in HDs 101 and 122. This was the second attempt at using helicopter capture to sample BHS in HD 122, thus we report on both years' data, below.

Table 1. Target and actual sample sizes, for winter 2016-2017 sampling of bighorn sheep. Numbers of captured yearlings and lambs, and adult ( $\geq 2$  yrs) females and males are provided.

HD & Herd name	Est. herd size	Recent (5 yr) demographic performance	Population objective	Target sample size	Information from handled animals				
					Date of capture	Total animals caught	Adult Females ( $\geq 2$ yrs)	Adult Males ( $\geq 2$ yrs)	Lambs & Yearlings
BHS HD 101: Kooconusa	25	Declining	$\geq 150$	18	2/10/2017-3/14/2017	2	2	0	0
BHS HD 102: Galton	90	Stable	$\geq 150$	29	1/12/2017	32	13	11	8
BHS HD 122: Clark Fork Cut-off	18	Declining	100-125	12	2/4/2016 & 2/14/2017	6*	6	0	0
BHS HD 482: Fergus	422	Declining	325	60	12/13/2016 & 2/21/2017	60	43	0	17
BHS HD 680: Missouri Breaks	458	Increasing	405-495	33	2/20/2017	33	26	0	7

\*In HD 122, we caught 4 BHS on 2/4/16 and 2 more on 2/14/2017.

### *BHS HD 101: Kooconusa*

The Kooconusa herd, also known as the Ural-Tweed herd, is located approximately 20 miles southwest of Eureka, in northwest Montana. Historically, this herd occupied both open bottom-land and the steep, rocky terrain along the Kooconusa River. However, with the creation of the Libby Dam and Lake Kooconusa in the 1970s, this herd's range was restricted to the steep, rocky, and forested eastern shore of Lake Kooconusa. Both the Kooconusa and Galton herds were historically considered native herds of "Trench sheep," a genetically distinct group of sheep whose larger range extends up through British Columbia. The Kooconusa herd has received two small augmentations: 5 rams from the National Bison Range were released within the Kooconusa herd in 1963, as well as 2 sheep from the Galton herd in 2006. It is also may

have some genetic connectivity to the Kootenai Falls herd (HD 100), which was founded by introductions of Sun River sheep. The Kooconusa herd contained 150-200 sheep in the 1960s, which fell to 20-25 animals in the 1970s, rebounded to 150-200 sheep in the 1990s, before declining and remaining low ever since. The herd currently contains approximately 25 animals. Habitat loss, predation, and inbreeding depression are all potential contributors to the low population size. There has been discussion of potentially augmenting this population with a small number of sheep from the Galton herd.

#### *BHS HD 102: Galton*

The Galton herd is located north of Eureka, in northwest Montana. The herd occupies a mix of private and public land, including the Woods Ranch Wildlife Management Area, and their range spans the US-Canadian border. Both the Kooconusa and Galton herds are believed to be native herds of “Trench sheep,” a genetically distinct group of sheep whose larger range extends up through British Columbia. Much of this herd’s home range is forested, so population counts are difficult with historic estimates being low (ranging from <10 to >60) and highly variable. Currently, 90 animals are estimated to live within this herd. The Galton herd has been of potential interest as a source population to augment the Kooconusa herd or the Wildhorse Island herd.

#### *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 in northwest Montana (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 dramatic decline in 2013, counts have been as low as 18 animals (Figure 2). This herd is currently comprised of just one group of animals. Data within this report combines information from 2017 (when 2 animals were captured) and 2016 (when 4 BHS were sampled) capture efforts. This herd will be augmented in the winter of 2017/18.

#### *BHS HD 482: Fergus & BHS HD 680: Missouri Breaks*

The Missouri Breaks, which encompass the rugged landscape surrounding the Missouri River in central Montana, are home to a metapopulation of bighorn sheep that span HDs 482 (Fergus), 680 (Missouri Breaks), 620 (Little Rockies), and 622 (Middle-Missouri Breaks). This metapopulation was founded through a series of introductions between the 1940s and 1980s. Sheep in HDs 482 and 680, which are across the river from one another, were founded in 1958-1961, with 45 animals from a combination of sheep from Sun River and the National Bison Range. In 1980, an additional 28 sheep from Sun River were released in this area. Sheep moved across the river, and both the Fergus and Missouri Breaks sub-populations steadily increased.

Bighorn sheep population objectives for HD 482 (including 300-350 sheep observed during aerial surveys) are designed to keep bighorn sheep habitat in healthy condition and sheep numbers well below carrying capacity to minimize the spread of diseases and to prevent or

minimize the occurrence of catastrophic die-offs. Despite a relatively low objective in relation to habitat, bighorn sheep in HD 482 have thrived, reaching a peak of 498 observed animals during the annual aerial survey in 2010. Supplemental forages in the form of agricultural fields juxtaposed with escape terrain help provide excellent habitat conditions for producing and growing sheep. Three bighorn sheep transplants have occurred from HD 482 since 2010, with 120 animals relocated to other areas of the state, primarily the Beartooth WMA. The last aerial survey was conducted in 2017, where the area biologist observed 377 sheep, including 113 rams, 184 ewes, and 81 lambs.

In HD 680, the average population count was 321 sheep between 1995-2015; in 2016, biologists counted 499 animals. Assuming observed sheep represent 60% of the total population, the actual population size may be over 700 animals. This population is fairly dispersed across its range with two connected sub-populations in the east and west half of the hunt district. There have been no observed/confirmed cases of pneumonia from this herd.

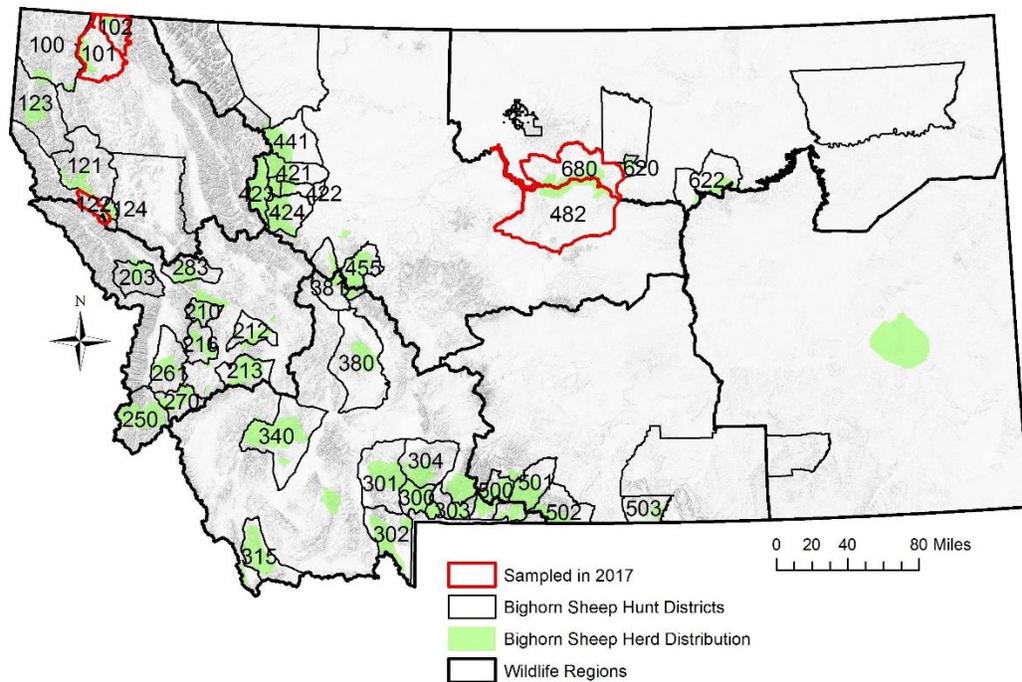


Figure 1. Map of (A) Montana’s bighorn sheep hunt districts (outlined and numbered) and associated herd distributions (green polygons). Herds within hunt districts outlined in red were visited for health sampling in 2016-2017.

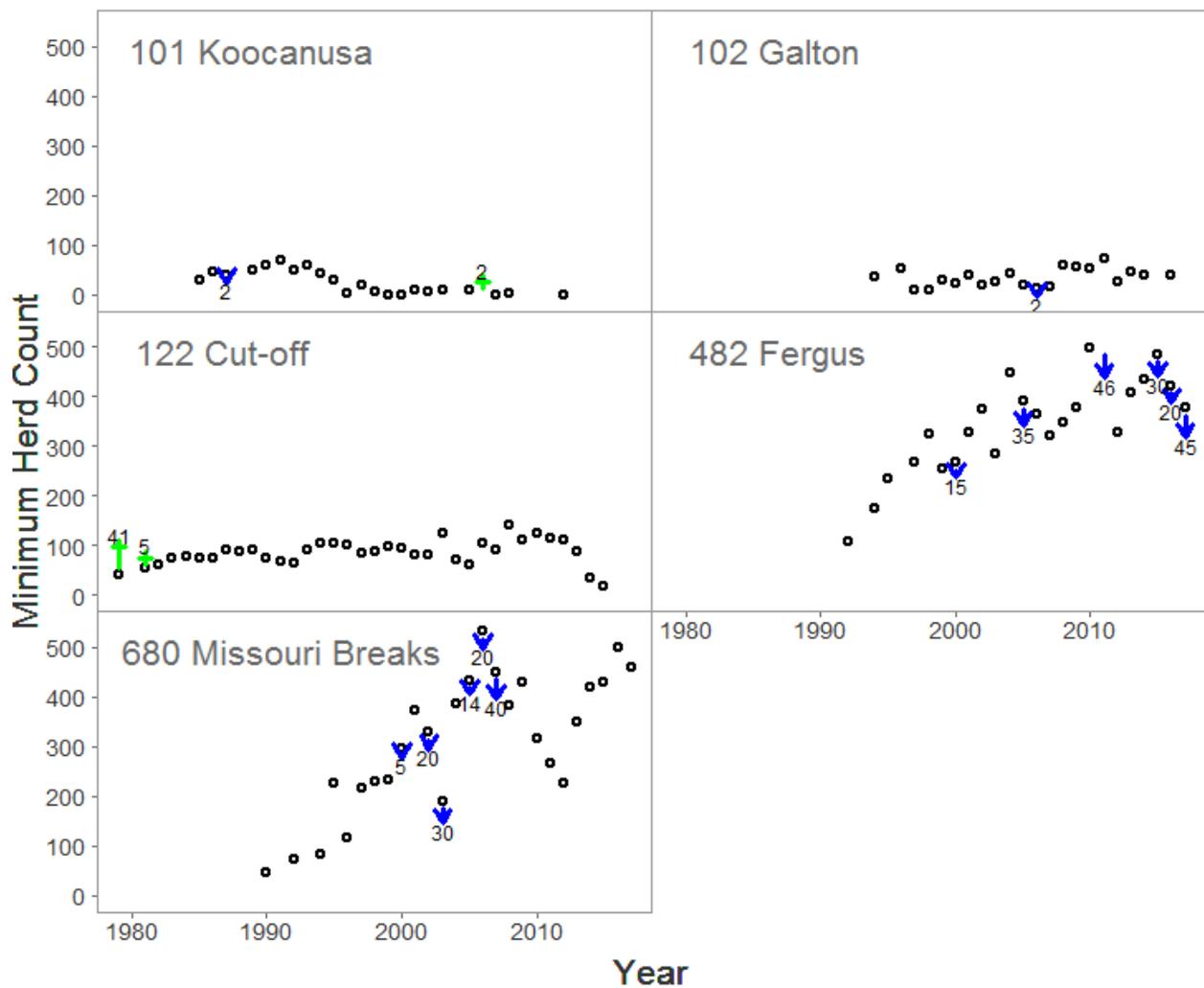


Figure 2. Minimum herd counts over time for the sampled bighorn sheep (HD 101, 102, 122, 482, and 680) herds. There have been no observed pneumonia die-offs within any of these herds. Historic translocations into and out of the herds are denoted with green plus and blue arrow symbols, respectively, with the number of animals moved noted. Sheep surveys were conducted December-August.

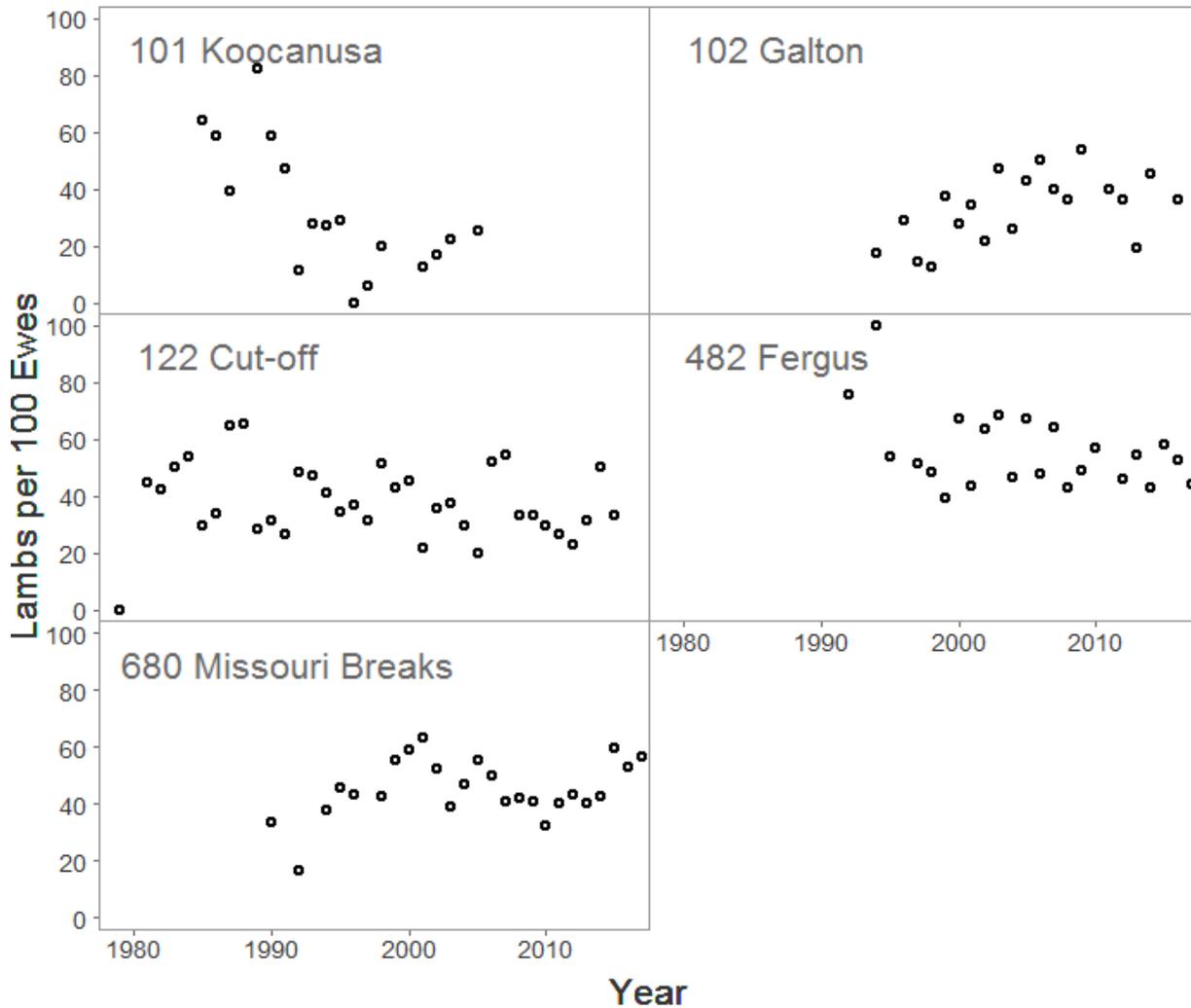


Figure 3. Minimum lambs per 100 ewes observed over time for the sampled bighorn sheep (HD 101, 102, 122, 482, and 680) herds. Sheep surveys were conducted December-August.

### **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 2017, Montana Fish Wildlife and Parks staff captured a total of 70 bighorn sheep from the four target populations via drop-net, ground darting, and helicopter net gunning (Table 1). In addition, 65 animals were captured via helicopter, sampled (n=60), and translocated out of the Fergus herd (HD 482), including 20 animals in December 2016 and 45 in February 2017. Target sample sizes for each population were defined to detect pathogens with at least 95% confidence, assuming pathogen seroprevalence was 10% at the population level. Work by Butler et al. (2017) showed that detection probabilities based on culture for many respiratory pathogens of interest are lower

than previously thought. To account for imperfect pathogen detection, we collected and tested duplicate tonsil swabs to increase our confidence in pathogen detection.

Efforts were made to broadly sample individuals within and across all known subgroups of each herd. We preferentially targeted adult female BHS 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 2 BHS captured in the Koocanusa herd (HD 101) to facilitate future helicopter capture and to obtain basic home range information.

### Sample Collection

Sheep captured as part of the Bighorn Sheep and Mountain Goat Herd Health Assessment Project 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, lactation status, a genetic sample, whole blood, blood serum, fecal samples, nasal and tonsil swabs (multiple tonsil swabs were collected and tested for all animals except the Galton herd (HD 102), where just one swab of each type was tested; swabs were placed in Tryptic soy broth medium and frozen at -80C until tested), and a sampling of any external parasites. A subset of these samples and data were collected from the ground-darted animals in HD 101. A variety of assays were employed to detect (1) a range of parasites and pathogens known to be relevant to BHS 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 (HD 101: December – May, by helicopter; HD 102: April – August, by helicopter and ground; HD 122: April by helicopter; HD 482: July - August by helicopter; HD 680: July - August, 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 was 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 from a swab of the bacterial growth from the primary streak zone of the culture plate. 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. Using Butler et al.'s (2017; Butler 2017) estimated detection probabilities for respiratory pathogens, we corrected raw estimates of exposure and infection rates by dividing the raw estimates by estimated detection probabilities and calculated corrected 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 101: *Koocanusa*

In February 2017, northwestern Montana received an extremely large amount of snow. Deep snow coupled with challenging capture terrain (steep, rocky, and forested), prevented the helicopter crew from capturing sheep in HD 101. However, regional wildlife staff were able to ground-dart 2 adult ewes between February 10-March 14, 2017, collect nasal and tonsil swabs, and fit the animals with radio-collars. Too few samples were collected to detect or accurately estimate the prevalence of pathogen infection or exposure in these herds. We did not detect any *Pasteurellaceae* or *Mycoplasma ovipneumoniae* on the two tonsil and nasal swabs (Figure 4 & 5), but this is not surprising given that our power to detect key respiratory pathogens was estimated to be <0.25 (Figure 5). Blood was collected, however it was not analyzed by the time of this report so it will be included in next year's summaries; fecal samples were also collected but they froze, making them unsuitable for analysis.

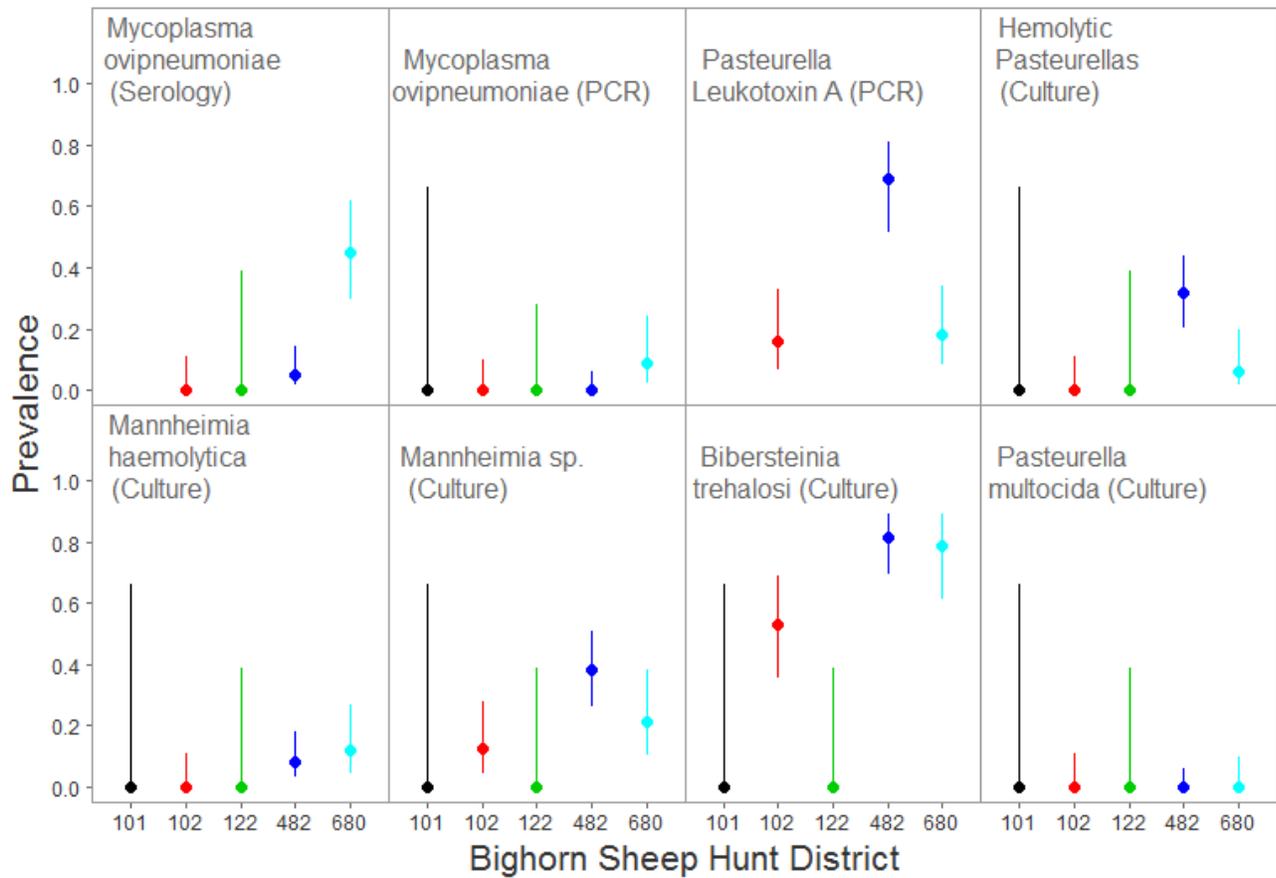


Figure 4. Summary of raw prevalence (proportion testing positive) and associated 95% binomial confidence intervals for respiratory pathogen exposures by herd. Data includes information from serology, PCR, and culture tests, as noted. This figure does not account for imperfect pathogen detection (see Figure 5).

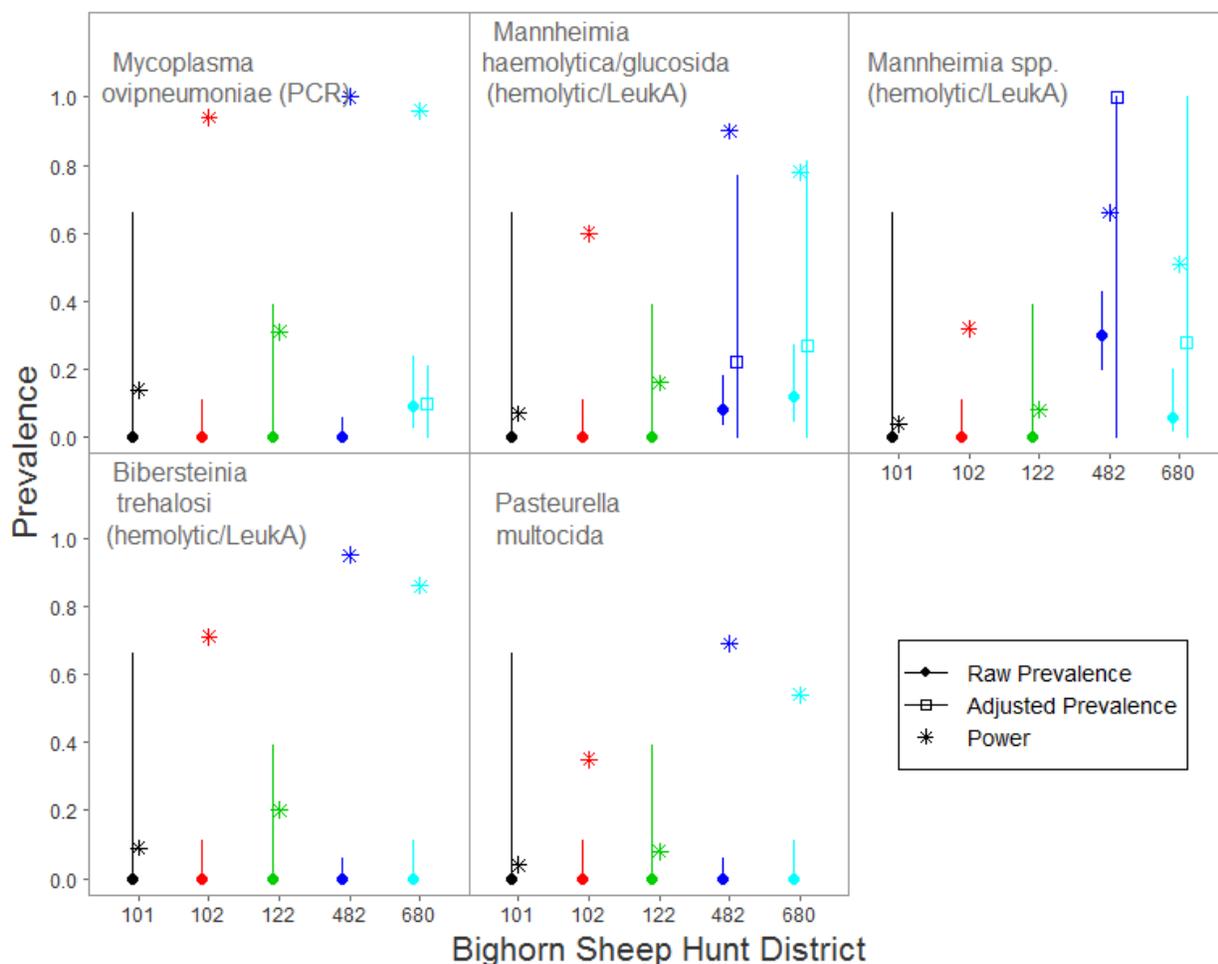


Figure 5. Summary of estimated raw (apparent) and adjusted (true) prevalence, and associated 95% confidence intervals, of select respiratory pathogens, accounting for imperfect detection probabilities (Butler 2017). Adjusted prevalences were calculated by dividing the raw estimates by estimated detection probabilities, and corrected 95% confidence intervals (in parentheses) were calculated using the delta method. We estimated the statistical power to detect the pathogen (denoted by the asterisk symbol) 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 for raw prevalences were estimated using the binomial distribution. Cultured *Mannheimia haemolytica* was assumed to be beta-hemolytic.

### BHS HD 102: Galton

On January 12, 2017, nearly 40 FWP staff and volunteers helped conduct a drop-net capture of 32 bighorn sheep from the Galton herd. We found no evidence from serology or PCR that this herd was infected with *Mycoplasma ovipneumoniae* (Figure 4), despite having >0.9 power to detect the pathogen at 10% prevalence via PCR (Figure 5). We sampled this herd prior to modifying our protocol to collect and test multiple swabs per animal; thus, despite a large sample size, we lacked sufficient power (i.e. <0.8) to detect most of the hemolytic/leukotoxigenic *Pasteurellaceae*. We did detect *Mannheimia* species and *Bibersteinia trehalosi*, but none of these were hemolytic. Although we failed to detect any hemolytic *Pasteurellaceae*, 16% of animals tested positive for the leukotoxin A gene from *Pasteurellaceae* cultured from tonsil swabs. In addition, we detected exposure to several moderate risk pathogens (Appendix I), including contagious ecthyma, anaplasma, bovine respiratory syncytial virus, ovine progressive pneumonia, *Leptospira sp*, and parainfluenza type-3, and detected active infections with both *Protostrongylus* and *Muellerius* lungworm species (Figure 6; Table 2).

In early March 2017, FWP collaborated with British Columbia to deploy store-on-board GPS collars on 5 Galton sheep. Blood samples and nasal swabs were collected and sent to labs by British Columbia's wildlife agency, and FWP has been given the results which will be incorporated in next year's report.

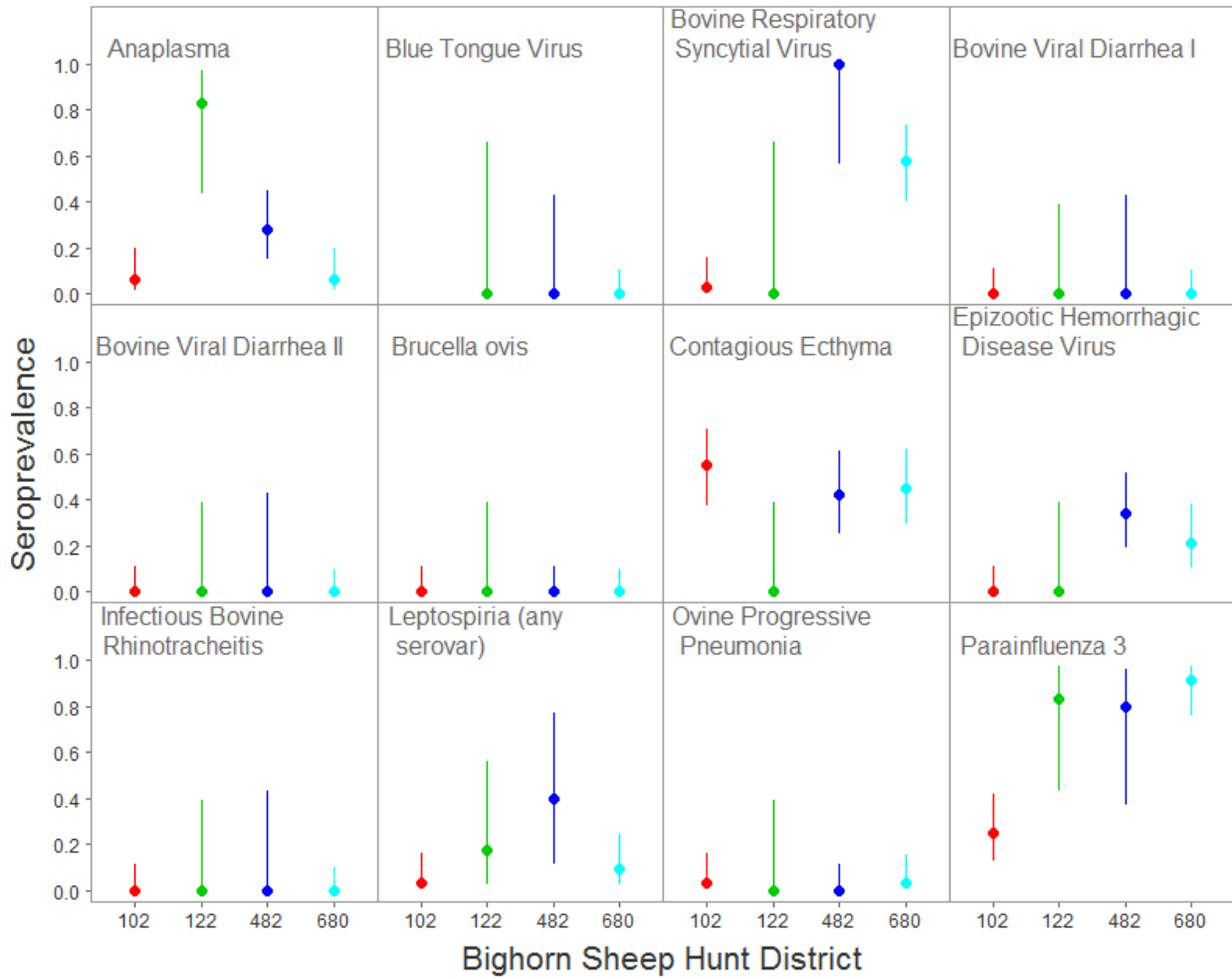


Figure 6. Summary of raw seroprevalence (proportion testing positive) and associated 95% binomial confidence intervals for pathogen exposures for moderate-risk pathogens, by herd.

Table 2. Summary of raw prevalence (proportion testing positive), 95% binomial confidence intervals (in brackets), and sample sizes for parasite exposures by herd.

Pathogen	Test Type	Perceived Riskiness	HD 101 BHS	HD 102 BHS	HD 122 BHS	HD 482 BHS	HD 680 BHS
<i>Psoroptes ovis</i>	Clinical	High	Not observed	Not observed	Not observed	Not observed	Not observed
Lungworm - Protostrongylus spp.	Baermann Fecal Float	Moderate	NA, n=0	0.33, [0.14, 0.61], n=12	1.0, [0.34, 1.0], n=2	NA, n=0	1.0, [0.7, 1], n=9
Lungworm - Muellerius spp.	Baermann Fecal Float	Moderate	NA, n=0	0.42, [0.19, 0.68], n=12	0, [0, 0.66], n=2	NA, n=0	0 [0, 0.3], n=9
Coccidia	Fecal Floatation	Low	NA, n=0	1, [0.76, 1], n=12	1, [0.34, 1], n=2	NA, n=0	0.33, [0.12, 0.65], n=9

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for sheep in HD 102 (Figure 7) 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 HD 102 fell below the published reference ranges for wild and domestic sheep.

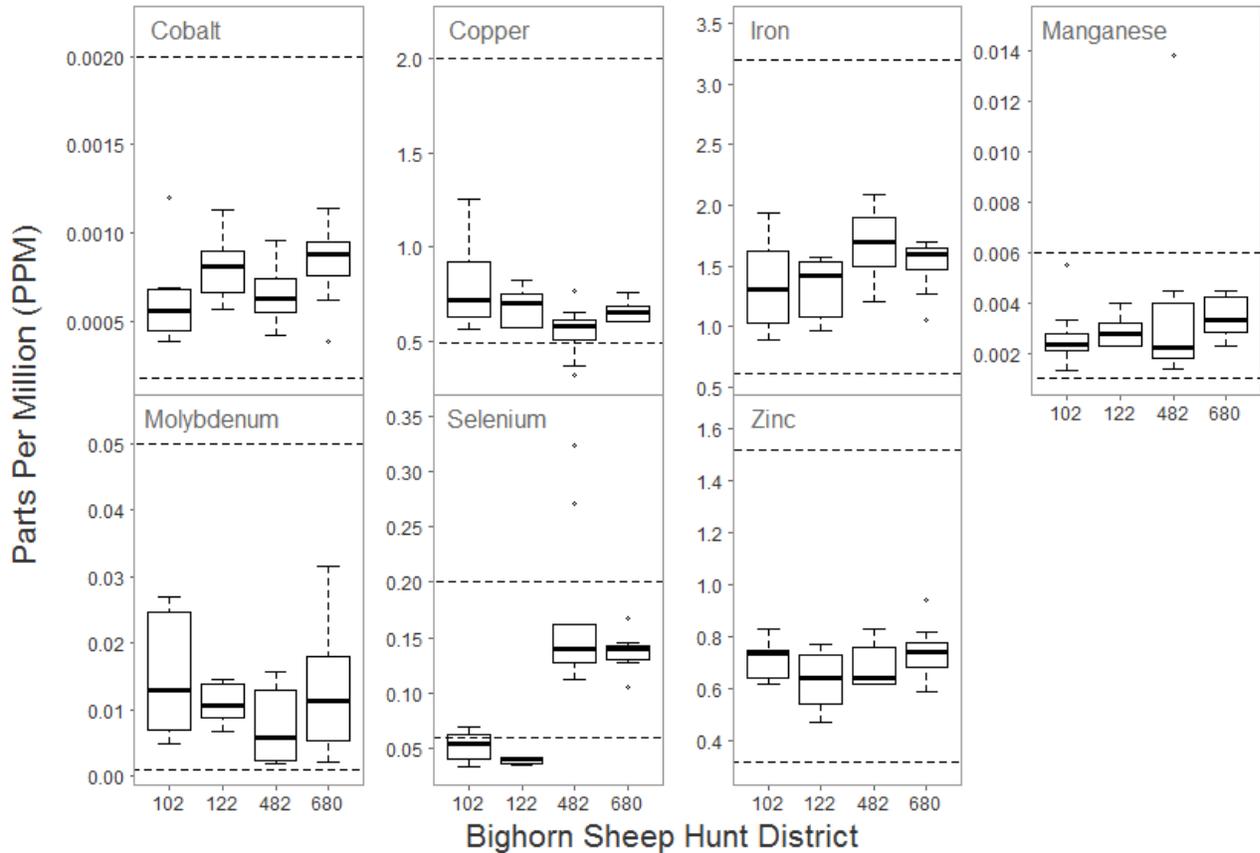


Figure 7. Boxplots of trace mineral concentrations in serum for cobalt, copper, iron, manganese, molybdenum, selenium, and zinc (displayed in parts per million, ppm) for each sampled herd. Horizontal dashed lines represent the minimum and maximum range of values observed in wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Sample sizes were as follows: HD 102 (n=10), HD 122 (n=6), HD 482 (n=10), and HD 680 (n=11).

Too few animals (n=2 adult ewes) were sampled for body condition indices, as captured by ultrasound-measured maximum rump fat and body condition score by palpation, to estimate an average herd-level index of condition (Figure 8).

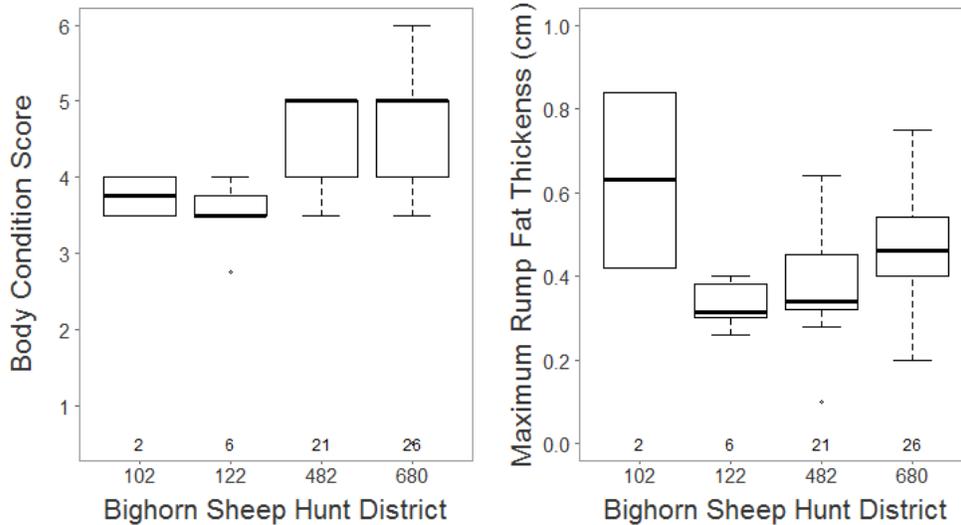


Figure 8. Boxplots of adult ( $\geq 2$  yrs) ewe body condition scores (scale of 0.5-6, following a protocol developed by Tom Stephenson, personal communication) assessed by palpation and ultrasound-measured maximum rump fat thickness (cm) for each sheep herd. Data only include samples collected during the months of January and February; our ability to assess lactation status becomes less reliable at this time, so the data do not distinguish based on lactation status. Sample sizes are noted.

#### BHS HD 122: Clark Fork Cut-off

Capture conditions remained difficult due to low densities of sheep, and steep, forested habitat in our second year of captures in HD 122. On February 14, 2017, the helicopter crew captured 2 adult ewes using a net gun (Table 1). We also captured 4 adult ewes from this herd on February 3-4, 2016. (Table 1). We combine both years of data here. Despite two sampling attempts, small sample sizes preclude accurately or precisely estimating the prevalence of pathogen infection or exposure in these herds. Our estimated power to detect key respiratory pathogens was too low (all  $< 0.32$ ) to comment on their absence with any certainty (Figure 5). However, we did detect exposure to several moderate-risk pathogens (Appendix I) in HD 122, including anaplasma, *Leptospira sp.*, and parainfluenza type-3 as well as active infections with *Protostrongylus* lungworm species (Figure 6; Table 2).

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for sheep in HD 122 (Figure 7) 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 HD 122 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.

Limited body condition indices, as captured by ultrasound-measured maximum rump fat and body condition score by palpation, are summarized for HD 122 in Figure 8. Overall, body

condition indices for adult ewes in January/February in HD 122 were comparable to those measured in the other herds.

#### BHS HD 482: Fergus

On December 13, 2016 30 bighorn sheep were captured via helicopter net-gun from HD 482 (Dog Creek area), 20 of which were translocated and released at Sheep Creek (HD 455). On February 21, 2017, another 45 sheep were similarly captured (from Dog Creek and the Castle Bluffs areas), translocated, and released in the Beartooth WMA (HD 455). Combining data from both captures gave us generally  $>0.8$  power to detect key respiratory pathogens at the  $\geq 10\%$  prevalence level within this herd (Figure 5). We found no evidence for active *Mycoplasma ovipneumoniae* infections by PCR, but 5% of animals tested positive for exposure on serology (Figures 5 & 6). We detected the presence of other moderate to high-risk respiratory pathogens (Appendix I), including: hemolytic *Pasteurellaceae*, Leukotoxin A+ *Pasteurellaceae*, *Bibersteinia trehalosi*, *Mannheimia haemolytica*, and *Mannheimia* species (Figures 5 & 6). In addition, we found evidence for exposure to contagious ecthyma, anaplasma, parainfluenza type-3, and bovine respiratory syncytial virus, *Leptospira sp.*, and epizootic hemorrhagic disease (Figure 6).

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, selenium, and zinc for sheep in HD 482 (Figure 7) 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, for adult females sampled in February are summarized in Figure 8. Overall, body condition indices for adult ewes in February in HD 482 were comparable to those measured in the other herds.

#### BHS HD 680: Missouri Breaks

On February 20, 2017, the helicopter crew net-gun captured 33 sheep in HD 680 (Table 1). Among these animals, we detected several high and moderate-risk respiratory pathogens (Appendix I), including: *Mycoplasma ovipneumoniae* by PCR and serology, hemolytic *Pasteurellaceae*, Leukotoxin A+ *Pasteurellaceae*, *Bibersteinia trehalosi*, *Mannheimia haemolytica*, and *Mannheimia* species (Figures 5 & 6). In addition, we found evidence for exposure to contagious ecthyma, anaplasma, ovine progressive pneumonia, parainfluenza type-3, bovine respiratory syncytial virus, *Leptospira sp.*, and epizootic hemorrhagic disease (Figure 6). This herd is also infected with *Protostrongylus* lungworm species (Table 2).

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, selenium, and zinc for sheep in HD 680 (Figure 7) 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, for adult females sampled in February are summarized in Figure 8.

Overall, body condition indices for adult ewes in February in HD 680 were comparable to those measured in the other herds.

## Discussion

In the winter of 2016-2017, we conducted health assessments on five bighorn sheep herds to help inform decisions regarding their use as potential source (BHS HDs 102 & 680; 482 was used as a source) or recipient herds (BHS HDs 101 and 122) for future translocations. Small sample sizes due to difficult capture conditions in HDs 101 and 122 limit our ability to adequately assess the pathogens present in these herds. The remaining three herds (HDs 102, 482, and 680) exhibited some evidence of infection with at least one pathogen of concern should they be mixed with naïve sheep (Figures 5, 6, & 7). The greatest diversity of high-risk agents involved in respiratory disease were detected in HD 680, including *Mycoplasma ovipneumoniae* (on PCR and serology in HD 680; only low prevalence on serology in HD 482) and Leukotoxin A positive and hemolytic *Pasteurellaceae*. Although we failed to detect the riskiest respiratory pathogens in HD 102, our statistical power to detect most of the hemolytic *Pasteurellaceae* was too low (<0.80; Figure 5) to confidently declare their absence from the herd. In addition, HDs 102, 482, and 680 all exhibited evidence of infection with contagious ecthyma, which when introduced to naive sheep, causes debilitating sores to form around the mouth, in some cases leading to death.

Trace element concentrations in each sampled herd appeared adequate according to previously published reference ranges in wild and domestic sheep, except for selenium, for which concentrations were low in HDs 102 and 122 (although small sample sizes in HD 122 limit the reliability of these estimates). Body condition indices for adult ewes in January/February were relatively similar between herds. Because we sampled most animals in January and February when lactation status is less reliably assessed, we were unable to summarize body condition in relation to lactation status, despite being known as an important predictor of body condition in other species elsewhere (Cook et al. 2013, Proffitt et al. 2016). 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. 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. (2017) point to *Mycoplasma ovipneumoniae* as a necessary agent involved in pneumonia outbreaks. However, it is worth noting that among 14 *Mycoplasma ovipneumoniae*-positive herds in Montana (n=7) and in Wyoming (n=7) (Butler 2017), at least half of the herds have exhibited adequate lamb recruitment and adult survival, and have recently been stable or increasing, despite some history of pneumonia epizootics. Conversely, we are unaware of populations that have a recent or more distant history of pneumonia epizootics in which *Mycoplasma ovipneumoniae* has not been detected, since diagnostic tools for *Mycoplasma ovipneumoniae* have become available. Therefore, based on the evidence accumulated to date, *Mycoplasma ovipneumoniae* 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 *Mycoplasma ovipneumoniae* and other co-infecting agents in wild sheep and goat respiratory disease.

We found evidence of *Mycoplasma ovipneumoniae*, the Leukotoxin A gene, and hemolytic *Pasteurellaceae* in HDs 482 and 680. The prevalence of *Mycoplasma ovipneumoniae* by serology and PCR in HD 482 appear to have declined from levels measured in 2014-2015 by Butler (2017). Neither herd has had a history of pneumonia or respiratory disease epizootics or known cases in individual sheep, and current population trends and recruitment ratios suggest large, stable populations. Both of these populations of sheep are remote and widely dispersed, so very localized issues with disease may be masked by aggregating estimates of pathogen prevalence and population dynamics over the entire HD. We failed to detect *Mycoplasma ovipneumoniae* in HD 102 both on serology and PCR, even with a high estimated power of detection (0.93). 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 *Mycoplasma ovipneumoniae* may be absent from HD 102.

*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. 2017, Walsh et al. 2012). Our sampling effort attempted to accommodate these low detection probabilities by testing multiple tonsil swabs per animal by culture. Despite this, we still faced low statistical power to detect some of these pathogens, particularly among herds in HDs 101, 102, and 122 (Figure 5). In all herds, we lacked sufficient power to detect *Pasteurella multocida*, which has exceptionally low detection probabilities on cultured tonsil swabs.

### **Management Implications**

Given that the bighorn sheep of HD 680 and 482 are infected with high-risk respiratory pathogens, where possible, we would recommend against using these herds as source populations for future translocations. These two herds are also positive for contagious ecthyma, and we would advise against translocating sheep positive for contagious ecthyma into a naïve herd. Although these two herds appear demographically robust, experience elsewhere suggests that moving potentially infected animals into a naïve herd could trigger epidemics. In both HD 482 & 680, translocation out of the herd has primarily been used as a tool to reduce population size. Ideally, if other tools such as increased ewe harvest could be accomplished, this might offer a safer approach to reducing population size while minimizing inadvertent pathogen spread. Ewe licenses are regularly issued for both these populations and have increased over time. However, hunting access on private land remains limited, which currently

hampers our ability to further reduce ewe numbers evenly across the herd. If translocations out of these herds are deemed too risky in the future, we need to work towards improving access and ewe harvest to keep the populations at objective.

The herds in HDs 482 and 680 are particularly interesting in that they remain demographically robust despite hosting some of the key respiratory pathogens, especially *Mycoplasma ovipneumoniae*. HD 482 has been repeatedly sampled from 2011-2017, both during translocations, as part of the State-wide Bighorn Sheep Research Project, and as part of a collaborative project with Washington State University's Agricultural Research Station (ARS). A more detailed analysis of how the prevalence and seroprevalence of *Mycoplasma ovipneumoniae* has varied over time may be helpful. Both HD 482 and 680 are large herds with considerable spatial structuring, which may buffer against consistent or widespread annual transmission. Further work on isolating and strain typing *Mycoplasma ovipneumoniae* from these herds may also prove insightful. Notably, despite obtaining multiple PCR-positive samples for *Mycoplasma ovipneumoniae* from HD 482, sequencing of isolates has failed to positively identify *Mycoplasma ovipneumoniae*, and instead has identified something that is more closely related to *Mycoplasma dispar*, a closely related cattle pathogen. Additional work on this is ongoing through WADDL and our cooperative project with Washington's ARS.

Due to challenging capture conditions, we only obtained small sample sizes in HD 101 & HD 122, which limits our ability to reliably assess the status of the pathogen community in these herds. This was our second attempt at capturing in HD 122. Currently, we are not scheduled to revisit these herds in 2018, although opportunistic ground darting or a drop-net capture in HD 101 could be considered in the future.

We did not detect *Mycoplasma ovipneumoniae* or the hemolytic *Pasteurellaceae* respiratory pathogens in samples collected from the bighorn sheep in HD 102, but as stated earlier, our power to detect the hemolytic *Pasteurellaceae* pathogens was insufficient to declare their absence (Table 3). We did detect evidence of the Leukotoxin A gene by PCR within this herd. 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. This herd is also positive for contagious ecthyma, which if transmitted via translocation to a naïve herd, could cause adverse effects.

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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

APPENDIX II: Supplementary figures and tables

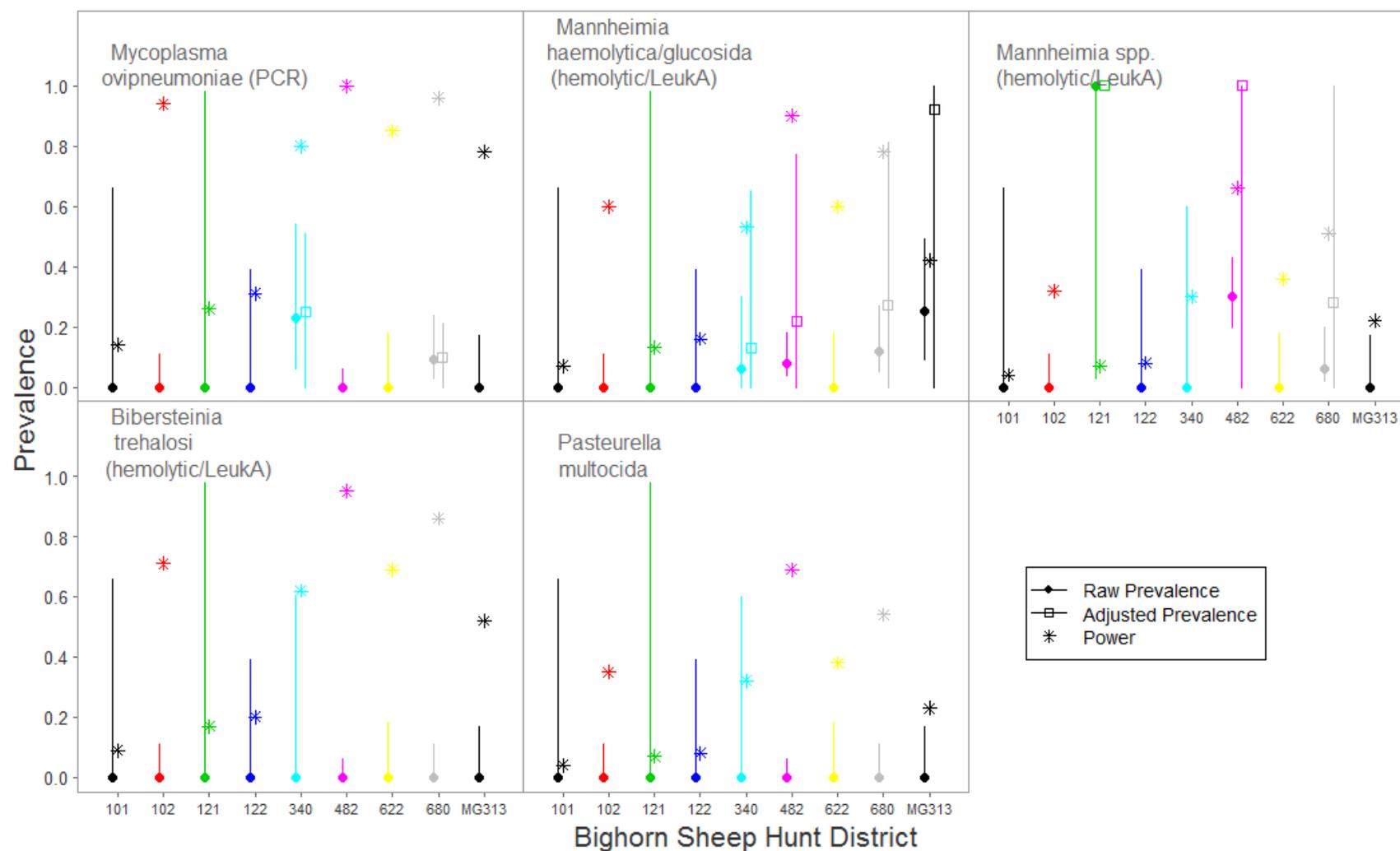


Figure A2.1. Figure of statistical power to detect, estimated raw (apparent) prevalence, and adjusted (true) prevalence of select, high-risk respiratory pathogens in herds sampled during 2016 & 2017 for P-R grant W-166-SI to Montana Fish, Wildlife & Parks, accounting for imperfect detection probabilities from Butler (2017). Adjusted prevalences were calculated by dividing the raw estimates by estimated detection

probabilities and corrected 95% confidence intervals 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 for raw prevalences were estimated using the binomial distribution. All data is from bighorn sheep, except that from MG313, which is from mountain goats caught in the Crazy Mountains (HD 313).