ASSESSING RESPIRATORY PATHOGEN COMMUNITIES AND DEMOGRAPHIC PERFORMANCE OF BIGHORN SHEEP POPULATIONS: A FRAMEWORK TO DEVELOP MANAGEMENT STRATEGIES FOR RESPIRATORY DISEASE

by

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ABSTRACT

Respiratory disease (pneumonia) is a persistent challenge for bighorn sheep (*Ovis canadensis*) conservation as sporadic epizootics cause up to 90% mortality in affected populations and are often followed by numerous years of low juvenile recruitment attributed to lamb pneumonia. Domestic sheep (*Ovis aries*) and domestic goats (*Capra aegagrus hircus*) are the origin of the disease and asymptomatically carry respiratory pathogens that cause respiratory disease when introduced to bighorn sheep. Pathogens that have been linked to respiratory disease in bighorn sheep include several species of bacteria in the *Pasteurellaceae* family and another bacterial species, *Mycoplasma ovipneumoniae*. Despite substantial efforts by management agencies to prevent contact between bighorn sheep and domestic sheep and goats, respiratory disease epizootics continue to affect bighorn sheep populations across much of their distribution with uncertain etiology. This study sought to investigate efficacy of diagnostic protocols in detecting *Pasteurellaceae* and *Mycoplasma ovipneumoniae* and generate sampling recommendations for different protocols, assess the distribution of these disease agents among 17 bighorn sheep populations in Montana and Wyoming and evaluate what associations existed between detection of these agents and demographic performance of bighorn sheep populations. Analysis of replicate samples from individual bighorn sheep revealed that detection probability for regularly-used diagnostic protocols was generally low (<50%) for *Pasteurellaceae* and was high (>70%) for *Mycoplasma ovipneumoniae*, suggesting that routine pathogen sampling likely mischaracterizes respiratory pathogen communities. Power analyses found that most pathogen species could be detected with 80% confidence at the population-level by conducting regularly-used protocols multiple times per animal. Each pathogen species was detected in over half of the study populations, and consideration of detection probability discerned that there was low confidence in negative test results for populations where the *Pasteurellaceae* species were not detected. 76% of study populations hosted Mycoplasma *ovipneumoniae* and *Pasteurellaceae* pathogens, yet a number of these populations were estimated to have positive population growth rates and recruitment rates greater than 30%. Overall, the results of this work suggest that bighorn sheep respiratory disease may be mitigated by manipulating population characteristics and respiratory disease epizootics could be caused by pathogens already resident in bighorn sheep populations.

ASSESSING RESPIRATORY PATHOGEN COMMUNITIES IN BIGHORN SHEEP POPULATIONS: SAMPLING REALITIES, CHALLENGES, AND RECOMMENDATIONS

Introduction

Respiratory disease has been a persistent problem for bighorn sheep restoration, with mortality during epizootics ranging from 10% to 90% of the affected population [1]. Epizootics affecting all age classes are often followed by multiple years of depressed lamb recruitment [2] as well as additional all-age epizootics of varying duration and severity [8,9]. The episodic nature of these disease outbreaks has led to hypotheses regarding the role of resident pathogens in a population versus the periodic introduction of novel pathogens [4,5]. Rigorous testing of these hypotheses has been limited by difficulties in accurately characterizing pathogen communities hosted by populations both before and after disease epizootics begin [5]. In addition, the poly-microbial nature of respiratory disease has made the identification of causative agents a challenging task. Recent research suggests *Mycoplasma ovipneumoniae* and *Pasteurellaceae* family pathogens (leukotoxigenic strains of *Mannheimia* or *Bibersteinia* genus organisms and potentially *Pasteurella multocida*) can play a role in the development of respiratory disease in bighorn sheep [6–10].

Wildlife managers regularly invest resources towards sampling bighorn sheep populations to assess what respiratory pathogens they host. Presence of respiratory pathogens in live animals is typically assessed by swabbing their nasal cavity and tonsillar crypts or oropharynx and testing those swabs for pathogens using culture or

polymerase chain reaction (PCR) methods. The results of these tests are used by wildlife managers to determine herd-health, disease-risk, and translocation decisions [11,12]. However, the detection probabilities (analogous to test sensitivity) of diagnostic protocols (i.e., the complete process by which samples are collected, handled, stored, and subjected to diagnostic tests) for live-sampled bighorn sheep may be low, particularly for *Pasteurellaceae* bacteria [13–15]; detection probabilities for *M. ovipneumoniae* diagnostic protocols have not been reported. Low detection probability has strong potential to lead to inaccurate conclusions of which pathogens are responsible for respiratory disease epizootics, and whether the outbreak was caused by introduction of novel pathogens or increased expression of pathogens already resident in the population (e.g., increased virulence or abundance within the host-pathogen complex, increased transmission rates, or increased proportion of susceptible individuals in the population) [4,16]. Additionally, low detection probability can lead to inappropriate or ineffective management decisions (e.g., translocations) when sampling does not detect important respiratory pathogens present in a bighorn sheep population.

The primary objectives of this study were to: 1) estimate detection probabilities for five bighorn sheep respiratory pathogens using various diagnostic protocols; 2) assess the precision with which respiratory pathogen prevalence can be estimated; 3) compare bias in prevalence estimates when detection error was, or was not, considered; and 4) evaluate the power to detect each pathogen in a population using different protocols with varying levels of pathogen prevalence, sampling intensity, and population sizes. Our goal is to provide guidance for bighorn sheep respiratory pathogen sampling and insights

regarding the potential for mischaracterization of pathogen communities in bighorn sheep populations.

Materials and Methods

Sample Collection and Diagnostic Protocols

One to four tonsil and nasal swabs were collected by trained personnel from bighorn sheep sampled in nine free-ranging populations in Montana, ten free-ranging populations in Wyoming, and one captive population in Wyoming between March 2013 and March 2016. Animals were captured between October 1 and March 31st each year using chemical immobilization, baited drop-nets, or helicopter net-gunning. Bighorn sheep were handled in accordance with IACUC permits held by Montana State University (#s 2011-17 and 2014-32), Montana Department of Fish Wildlife & Parks permit number 2016-005 or Wyoming Game & Fish Department (WGFD) permit number 854.

Swab samples were collected using sterile polyester-tipped applicators (Puritan #25-806 1PD, Guilford, Maine, USA). Collection of tonsil swabs was aided by the use of a lighted swab extender and tongue depressors to better target the tonsils and tonsillar crypts. Tonsil swab samples were assessed for the presence of *Pasteurellaceae* pathogens and nasal swabs were assessed for presence of *M. ovipneumoniae,* with the exception of one capture and sampling event where nasal swabs were also assessed for presence of *Pasteurellaceae* pathogens. Replicate sampling of individual animals was generally conducted using different diagnostic protocols, but was conducted by repeating the same

protocol twice for a subset of individuals. Samples were labeled so that replicates from the same individual animal could not be identified by laboratory personnel. The same set of diagnostic protocols was not used for all sampling events due to logistical constraints including: availability of trained personnel, specialized equipment, and transport media; agency responsible for the sampling event; and availability of laboratory personnel for protocols requiring diagnostic tests not offered by a fee-for-service laboratory (non-FFS).

All *Pasteurellaceae* pathogens were detected using one set of five diagnostic protocols and *M. ovipneumoniae* was detected using a different set of three diagnostic protocols (Table 1). Diagnostic tests offered by a fee-for-service (FFS) laboratory (Washington Animal Disease Diagnostic Laboratory-WADDL) were used to detect and identify respiratory pathogens for four protocols (FFS protocols). Diagnostic tests conducted at a non-FFS diagnostic laboratory (Wyoming Game and Department Wildlife Health Laboratory-WGFD) were used to detect and identify respiratory pathogens for three protocols (non-FFS protocols). A non-FFS diagnostic test also was conducted at WADDL as part of protocol development. For *Pasteurellaceae*, all FFS protocols detected pathogens by culture. The FFS *M. ovipneumoniae* protocol detected this pathogen by PCR. Non-FFS protocols used PCR (sometimes in conjunction with culture) to detect each pathogen, with the exception of *P. multocida*, which was only detected by culture.

Table 1.1. Summary of diagnostic protocols used in this study to detect respiratory pathogens in bighorn sheep. One set of protocols was used to detect *Pasteurellaceae* pathogens and a different set was used to detect *Mycoplasma ovipneumoniae.*

Pathogen Group	Protocol	Media[2]	Diagnostic Lab[3]	Diagnostic Test[2]
Pasteurellaceae				
	TSB	TSB	WADDL	Culture
	Port-A-Cul	Port-A-Cul™	WADDL	Culture
	Plated Culture	CBA + TSB	WADDL	Culture
	Plated PCR[1]	CBA[4]	WGFD	PCR
	Wyoming	CBA & Port-A-Cul™	WGFD	Culture + PCR
Mycoplasma ovipneumoniae				
	TSB	TSB	WADDL	PCR
	qPCR	None	WADDL	PCR
	Wyoming	Port-A-Cul™ + TSB-1	WGFD	PCR

[1] The Plated PCR protocol did not assess presence of *Pasteurella multocida.*
[2] Where two media or test types are listed, a "&" symbol indicates both where employed simultaneously and independently and a "+" symbol indicates a sequence. TSB is an abbreviation for tryptic soy broth with 15% glycerol, CBA is an abbreviation for Columbia Blood Agar ™, and TSB-1 is an abbreviation for modified tryptone soy broth.

Pasteurellaceae Protocols. *Pasteurellaceae* pathogens assessed in this study included beta-hemolytic or leukotoxigenic strains of *Bibersteinia trehalosi (B. trehalosi)*, *Mannheimia haemolytica (M. haemolytica)*, and *Mannheimia* spp., as well as *Pasteurella multocida (P. multocida).* Presence of *Pasteurellaceae* pathogens was assessed using five diagnostic protocols explained below and described in detail in Appendix A.

Wyoming protocol: Two tonsil swabs were collected from each animal, one was immediately inoculated onto a Columbia Blood Agar (CBA) culture plates with 5% sheep blood (Hardy Diagnostics, Santa Maria, California, USA), and one was stored at approximately 4°C in a 10 mL Port-A-Cul™ transport media tube for approximately 6 hours before inoculating a second CBA plate which was incubated at 37°C in 5% CO_2. *Pasteurellaaceae* pathogens on CBA plates were identified at WGFD using a

combination of culture and PCR tests. Presence of *P. multocida* was assessed solely by culture.

TSB protocol: Swabs were each placed into a vial of tryptic soy broth with 15% glycerol (TSB; Hardy Diagnostics, Santa Maria, California, USA) immediately after collection. The TSB vials were immediately frozen (approximately -20^{o}C), and later shipped overnight on dry ice to WADDL where they were assessed for presence of any *Pasteurellaceae* pathogens using FFS culture tests.

Port-A-Cul protocol: Swabs were immediately placed into 10 ml Port-A-Cul ™ transport media tubes (BD, Sparks, Maryland, USA), and kept cool until being shipped on ice overnight to WADDL within 48 hrs. Samples were assessed immediately upon arrival at WADDL for presence of any *Pasteurellaceae* pathogens using FFS culture tests.

Plated Culture protocol: Tonsil swabs were used to immediately inoculate a CBA plate which was then incubated at 37^{o}C in 5% CO_2. After 24 hours, a swab of the primary streak zone and phenotypically distinct (though unidentified) colonies was collected from each CBA plate, placed into TSB, and immediately frozen at approximately -20^{o}C. Vials were shipped overnight on dry ice to WADDL where they were assessed for presence of any *Pasteurellaceae* pathogens using FFS culture tests.

Plated PCR protocol: Following completion of the Plated Culture protocol, the inoculated CBA plates were incubated for approximately 24 additional hours at 37^{o}C in 10% CO_2. Then bacterial growth was removed from the surface of the CBA plate, suspended in 4ml of PBS and frozen at -20^{o}C until PCR tests were conducted at WGFD. Presence of *P. multocida* was not assessed by this protocol.

Mycoplasma ovipneumoniae Protocols. Presence of *M. ovipneumoniae* was assessed using the three diagnostic protocols explained below and described in detail in Appendix A:

Wyoming Protocol: Swabs were immediately placed into a 10 mL Port-A-Cul™ transport media tube or Amies media without charcoal in 10 mL culture tubes and kept at approximately 4 ^{o}C until being placed in modified tryptone soy broth (TSB-1) within 72 hours of collection. The TSB-1 broth was incubated at 37^{o}C in 5% CO_2 for 48 hours and tested for presence of *M. ovipneumoniae* at WGFD using PCR.

TSB protocol: Swabs were immediately placed into TSB vials after sample collection. The TSB vials were immediately frozen (approximately -20^{o}C), and later shipped overnight on dry ice to WADDL where they were assessed for *M. ovipneumoniae* presence using a FFS PCR test.

qPCR protocol: Swabs were immediately sealed in a sterile vial after sample collection and kept at approximately -20^{o}C. Vials were later shipped overnight on dry ice to WADDL where they were tested for *M. ovipneumoniae* using quantitative PCR (qPCR) as part of new protocol development (i.e., this is a non-FFS diagnostic test).

Pathogen Classification

Pasteurellaceae organisms were classified based on hemolysis on CBA or the presence of the leukotoxin gene (lktA) as indicated by PCR; non-hemolytic/non-leukotoxigenic strains in the *Mannheimia* or *Bibersteinia* genera were not considered in this analysis. To make results obtained from culture and PCR tests comparable, beta-hemolysis and presence of lktA were considered synonymous in the categorization of

organisms, as beta-hemolysis in culture is well correlated with presence of the lktA in *Pasteurellaceae* organisms [17]. Diagnostic tests do not consistently distinguish among some species in the *Mannheimia* genus [18]. For this analysis *Mannheimia glucosida* was included within the *M. haemolytica* classification, as the available PCR primers amplify the target genes of both species [7]. Other *Mannheimia* genus pathogens, including *Mannheimia ruminalis* and unidentified *Mannheimia species*, were combined into a single group for analysis (*Mannheimia* spp.), as isolates currently identified as *M. ruminalis* by WADDL were likely classified as unidentified *Mannheimia species* by WGFD (Wyoming Game & Fish Department unpublished data). *B. trehalosi, P. multocida* and *M. ovipneumoniae* were classified as reported by the diagnostic laboratory.

Estimating Detection Probability and Prevalence

Single-species, single-season occupancy modeling [19] using package "unmarked" [20] in program R [21] was used to independently estimate detection probability (ρ) and prevalence (ψ) of each respiratory pathogen within each population, where individual bighorn sheep constituted sampling sites and the protocols that were conducted constituted the encounter history for each animal. For each pathogen, two model structures were considered for detection probability: detection probability was either held constant across all protocols or allowed to vary freely across protocols. To assess prevalence, individuals were assigned to populations based on the management unit (defined by the overseeing wildlife agency) in which they were sampled. Three competing model structures were evaluated to describe prevalence of each pathogen: 1)

constant across all populations; 2) variable among populations but not years; and 3) variable among populations and years (i.e., population-years). All combinations of detection and prevalence structures resulted in a total of six analogous candidate models for each pathogen.

To reduce the number of model parameters and avoid model-convergence issues related to estimating logit-scaled parameters at a boundary, data from population-years where a respiratory pathogen was undetected and/or fewer than five animals were sampled were omitted from analysis. Adequate model convergence was confirmed by verifying that each model's condition number was less than 10^4 [22]. An attempt was made to estimate overdispersion ($\hat{c}$) by applying the MacKenzie and Bailey goodness-of-fit test [23] to the full model ($\rho \sim$ Protocol, *Prevalence* ~ Population-Year) for each pathogen; however, the expected values that generate the χ^2 statistics for this test were often less than one, indicating that the data were too sparse to accurately estimate $\hat{c}$ [24]. The stability of detection probability estimates for each pathogen was assessed by fitting the full model to two subsets of data collected by different personnel in different states (Montana or Wyoming) and comparing the resulting estimates of detection probability to those obtained from analysis of the complete dataset (Appendix B).

Assessing Power to Detect Pathogens at the Population-Level

Estimates of pathogen detection probability for different protocols can be used to estimate the minimum sampling effort required to determine presence of respiratory pathogens in a population. In this context, the power of a protocol to detect a pathogen in

an infected population of bighorn sheep (i.e., the probability of detecting a pathogen in at least one animal) can be derived from the estimate of detection probability for that protocol. Bayes theorem was used to derive the power to detect each pathogen in a bighorn sheep population given the protocol used, number of animals sampled and number of times the protocol was conducted per animal, pathogen prevalence, and population size. A full description of the derivation is provided in Appendix C.

The power to detect each of the pathogens in at least one animal during a sampling event was calculated (given the detection probability distribution for a specified pathogen and protocol) while varying the number of animals sampled (from 1 to 100), the number of times the protocol was conducted per animal (1, 2 or 3), pathogen prevalence (0.10, 0.30, or 0.50), and population size (25, 50, 100, or 200). These findings were used to identify sampling methodologies (i.e., combinations of diagnostic protocols, animals sampled, and number of times the protocols are conducted per animal) predicted to result in adequate detection power for each pathogen (defined as $\geq$0.80 following Ellis *et al.* 2014 [25]). To illustrate the effects of specific variables on the power to detect pathogens, variables were constrained to subjective "default" values that we defined. Unless otherwise specified, the "default" number of animals sampled was set at 25, population size was set at 100, number times a diagnostic protocol was conducted per animal was one, and the default protocol was the TSB protocol. Default prevalence was set at 0.10, which was considered a realistic value to represent low prevalence based on estimates obtained in this study. Protocols whose detection probability was estimated to be zero or

one (*Mannheimia* spp.-Port-A-Cul protocol, *B. trehalosi*- Port-A-Cul protocol, *Pasteurella multocida*-Plated Culture protocol) were not considered in this assessment.

Results

Sampling Effort

A total of 2093 *Pasteurellaceae* diagnostic tests were conducted for 476 bighorn sheep and a total of 768 *M. ovipneumoniae* diagnostic tests were conducted for 469 bighorn sheep. The TSB and Plated Culture (*Pasteurellaceae)* protocols were conducted twice for 165 and 61 animals, respectively; all other *Pasteurellaceae* protocols were conducted once per animal. Various combinations of two *Pasteurellaceae* protocols were conducted on 178 animals; three *Pasteurellaceae* protocols were conducted on 108 animals, four *Pasteurellaceae* protocols were conducted on 23 animals, five *Pasteurellaceae* protocols were conducted on 26 animals, and six *Pasteurellaceae* protocols were conducted on 45 animals. Among *M. ovipneumoniae* protocols, only the TSB protocol was conducted more than once per animal, and was conducted twice for 117 animals. Various combinations of two *M. ovipneumoniae* protocols were conducted on 278 animals and three *M. ovipneumoniae* protocols were conducted on 11 animals.

Pathogen Detection Summaries

The total number of detections for individual pathogen species varied from 44 *P. multocida* detections to 152 *M. ovipneumoniae* detections (Table 1.2). Considering animals where multiples diagnostic protocols were conducted and the respective pathogens were detected, *M. haemolytica* was detected by more than one diagnostic test

30% of the time (14 of 46 animals), *Mannheimia* spp. 7% (9 of 128), *B. trehalosi* 37% (22 of 60), *P. multocida* 2% (1 of 42), and *M. ovipneumoniae* 60% of the time(35 of 59). When the targeted pathogens were detected in a population-year, the minimum estimates of naïve prevalence (i.e., the proportion of animals from a population where the pathogen was detected in a given year) varied from 0.03 to 0.17 among the pathogens and the estimates of maximum naïve prevalence varied from 0.44 to 1.00 (Table 1.2).

Model Selection

The top-ranked model for *Mannheimia* spp., *B. trehalosi,* and *P. multocida* detection probability (ρ) included diagnostic protocol as a predictor. The top-ranked model for *M. ovipneumoniae* and *M. haemolytica* estimated a constant detection probability across all protocols (Table 1.3). However, the 2nd-ranked model for both these pathogens included diagnostic protocol as a predictor and, given their relatively modest ΔAIC_c scores (2.11 and 3.25, respectively), provided some evidence that detection probability is not identical for all protocols. The top-ranked model for all pathogen datasets allowed the estimate of prevalence to vary across populations (*Mannheimia* spp. and *B. trehalosi*) or across populations and years (*M. ovipneumoniae, M. haemolytica,* and *P. multocida).* For all pathogen datasets, the full model (ρ ~ Protocol, *Prevalence* ~ Population-Year) was either the top-ranked model or the second-ranked model. Provided this level of support as well as the objective to describe estimates of detection probability for the different combinations of pathogens and protocols, parameter estimates obtained from the full model for each pathogen were interpreted, even though the full model was not always the most parsimonious.

Table 1.2. Sampling results summaries and naïve prevalence estimates for five bighorn sheep respiratory pathogens.

	Mycoplasma ovipneumoniae	*Mannheimia haemolytica*[1,2]	*Mannheimia species.*[2]	*Bibersteinia trehalosi*[2]	*Pasteurella multocida*
Diagnostic tests[3]	768	1268	1268	1268	1057
(# Positive)	(152)	(82)	(141)	(94)	(44)
Animals sampled	469	476	476	476	476
(# Positive)	(118)	(66)	(132)	(67)	(43)
Range of naïve prevalence[4]	0.051-0.828	0.062-0.44	0.167-0.615	0.029-1.00	0.059-0.875

1. This classification includes isolates identified as *Mannheimia glucosida* (n=8).
2. Only beta-hemolytic or leukotoxigenic strains are summarized.
3. The *total* number of individual *Pasteurellaceae* diagnostic tests is 2093, not the apparent sum, because an individual culture test assesses presence of all four targeted *Pasteurellaceae* pathogens.
4. Naïve prevalence is estimated as the proportion of animals from a sampled population in a given capture season in which the pathogen was detected. Population-years where less than 5 animals were sampled and where a pathogen was not detected are not included.

Detection Probability Estimates

Detection probability for *M. ovipneumoniae* was greater than 0.60 for all diagnostic protocols that were evaluated and detection probabilities of all FFS diagnostic protocols (i.e., protocols that entailed shipping samples to WADDL for diagnostic testing) for *Pasteurellaceae* pathogens were less than 0.50 (Figure 1.1). No single diagnostic protocol used to detect the *Pasteurellaceae* pathogens resulted in estimated detection probabilities greater than 0.50 for all four targeted *Pasteurellaceae* pathogens. The FFS *Pasteurellaceae* protocols generally detected the targeted pathogens less effectively than the non-FFS diagnostic protocols:

range $\hat{p}_{commercial}$: $0.0 - 0.44$, range $\hat{p}_{non-commercial}$: $0.19 - 0.96$).

Table 1.3. AIC_c rankings of occupancy models with different explanatory variables for pathogen detection probability and prevalence. Among these models, estimates of detection probability (ρ) for each respiratory pathogen were either 1) held constant or 2) allowed to vary by diagnostic protocol. Prevalence estimates of the respiratory pathogens were either 1) held constant; 2) allowed to vary among populations, or 3) allowed to vary among years within a population (i.e., population-years). Top-ranked models for each pathogen dataset are bolded and underlined.

	Mycoplasma ovipneumoniae		*Mannheimia haemolytica*		*Mannheimia species*		*Bibersteinia trehalosi*		*Pasteurella multocida*	
Model Structure	K	ΔAIC_c	K	ΔAIC_c	K	ΔAIC_c	K	ΔAIC_c	K	ΔAIC_c
ρ ~ Protocol *Prevalence* ~ Population-Year[1]	21	2.11	21	3.25	24	7.99	13	2.50	**10**	**0.00**
ρ ~ Protocol *Prevalence* ~ Population	16	59.55	17	10.37	**19**	**0.00**	**11**	**0**	9	9.83
ρ ~ constant *Prevalence* ~ Population-Year	**19**	**0.00**	**17**	**0.00**	20	204.93	9	85.55	7	47.28
ρ ~ constant *Prevalence* ~ Population	14	57.49	13	7.07	15	194.96	7	82.72	6	61.30
ρ ~ protocol *Prevalence* ~ constant	4	87.04	6	4.62	6	20.30	6	39.89	5	21.39
ρ ~ constant *Prevalence* ~ constant	2	86.91	2	7.74	2	177.22	2	184.07	2	82.99

[1.] Full model from which reported estimates of detection probability and prevalence were obtained.

Mycoplasma ovipneumoniae. The estimated detection probability of *M. ovipneumoniae* using the Wyoming protocol was highest ($\hat{p}$ = 0.85, 95% CI: 0.66-0.94), however, the TSB protocol was comparable ($\hat{p}$ = 0.72, 95% CI: 0.62-0.81). Detection probability using the qPCR protocol was also comparable and estimated at 0.64 (95% CI: 0.32-0.87).

Mannheimia haemolytica. All protocols were relatively poor at detecting *M. haemolytica*, with estimated detection probabilities ranging from 0.10 (95% CI: 0.01-0.48) to 0.45 (95% CI: 0.24 – 0.68). Among the FFS protocols used to detect *M. haemolytica*, the Plated Culture and TSB protocols were comparable and performed best, with estimated detection probabilities of 0.30 (95% CI: 0.16-0.51) and 0.27 (95% CI: 0.16-0.40), respectively. Among non-FFS protocols, The Wyoming protocol had the highest estimated detection probability for *M. haemolytica* ($\hat{p}$ = 0.45, 95% CI: 0.24-0.68).

Mannheimia species. The Plated PCR protocol had the highest estimated detection probability for *Mannheimia spp.* ($\hat{p}$ = 0.95, 95% CI: 0.74-0.99). All other protocols were poor at detecting *Mannheimia spp.* ($\hat{p}$ < 0.19), with the TSB protocol having the highest estimated detection probability among the commercially available protocols ($\hat{p}$ = 0.12, 95% CI: 0.08-0.16).

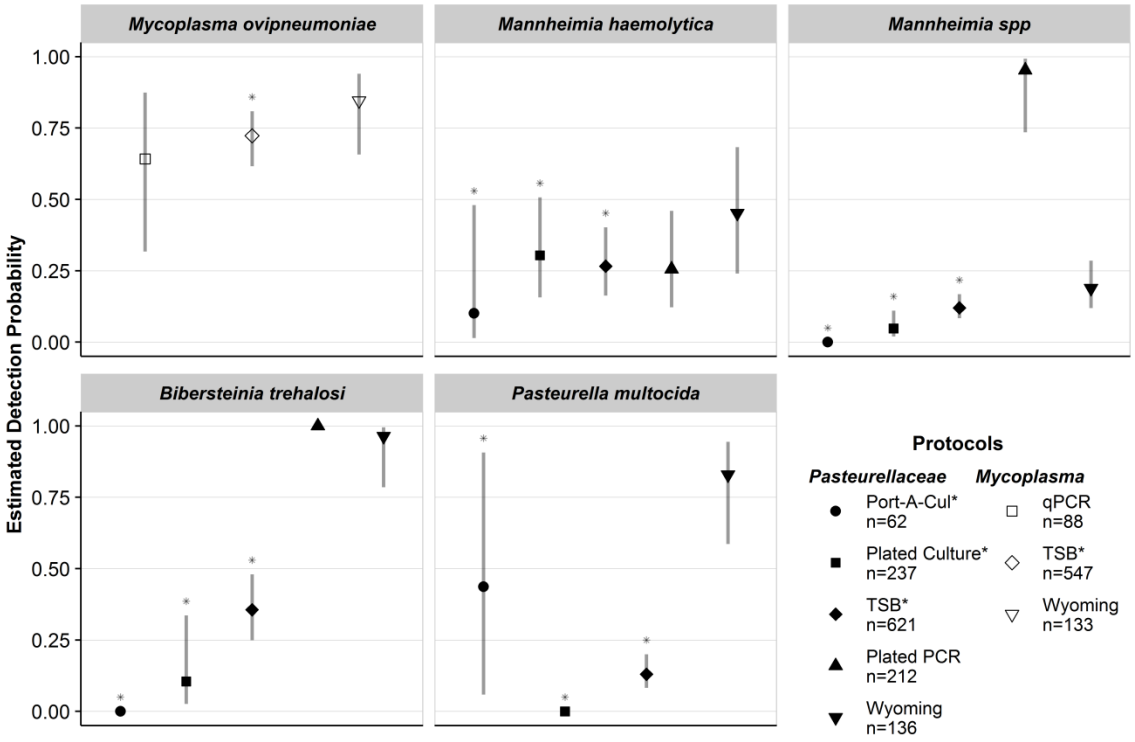


Figure 1.1. Estimated detection probabilities and 95% confidence intervals for five bighorn sheep respiratory pathogens. One set of protocols was used to detect the four Pasteurellaceae organisms (shaded) and a separate set of protocols was used to detect *Mycoplasma ovipneumoniae* (not shaded). Detection probabilities for *Mannheimia haemolytica*, *Mannheimia* spp., and *Bibersteinia trehalosi* are for beta hemolytic or leukotoxigenic strains. Protocols that used diagnostic tests through a fee-for-service laboratory are indicated with an asterisk (*) in the legend and above the upper confidence limit. The total number of samples assessed using each protocol is indicated in the legend.

<u>*Bibersteinia trehalosi.*</u> The Wyoming protocol was good at detecting *B. trehalosi*, with an estimated probability of 0.96 (95% CI: 0.79-0.99). The detection probability for the Plated PCR protocol was estimated to be 1; however, this estimate is unreliable because *B. trehalosi* was only detected in two animals (out of 211) where the Plated PCR protocol was conducted. The highest estimated detection probability among protocols that used FFS diagnostic protocols for *B. trehalosi* was 0.36 (95% CI: 0.25-0.48) and was achieved using the TSB Protocol.

Pasteurella multocida. The highest detection probability for *P. multocida* was realized using the Wyoming protocol ($\hat{p}$ = 0.83, 95% CI: 0.59-0.94). Among FFS protocols, the estimated detection probability was highest for the Port-A-Cul protocol, 0.43, however precision of this estimate was poor (95% CI: 0.06-0.91). Estimated detection probabilities for *P. multocida* using the TSB and Plated Culture protocols were 0.13 (95% CI: 0.08-0.20) and 0 (inestimable 95% CI), respectively.

Stability of Detection Probability Estimates. Independent analysis of subsets of data collected by different personnel in different states indicated estimates of detection probability were consistent between the subsets for all combinations of pathogens and protocols, with the exception of the TSB protocol for *Mannheimia* spp. (Appendix B). For this pathogen-protocol combination, analysis of the subset of data from animals sampled in Montana resulted in an estimated detection probability of 0.01 (95% CI: 0.00-0.06), whereas analysis of the subset of data from animals sampled in Wyoming resulted in an estimated detection probability of 0.31 (95% CI: 0.21-0.43). The differences between these estimates and the corresponding estimate obtained from the complete dataset were 0.11 (Montana subset) and 0.19 (Wyoming subset). For all other pathogen-protocol combinations, the difference between the detection probability estimates obtained from the subsets of data and the estimate obtained from the complete dataset was small ($\mu_{\text{difference}}$ = 0.04) and 95% confidence intervals overlapped substantially (Appendix B, Figure B1).

Pathogen Prevalence

Prevalence estimates and associated 95% confidence intervals are presented from four different sampling events that best represent a range of reasonable sampling intensities; these sampling events include the Hilgard population in 2013/2014, the Perma-Paradise population in 2014/2015, the Highlands population in 2015/2016, and the Stillwater population in 2014/2015. The number of animals from each population that were sampled using each diagnostic protocol is shown in Appendix B (Table B1, Table B2). Among these populations, pathogen prevalence estimates ranged from 0 (not detected in the sampling event) to 1. Excluding these extremes, estimates of pathogen prevalence ranged from 0.14 to 0.91 (Figure 1.2).

When only FFS diagnostic protocols were used (i.e., no PCR tests were conducted) to detect *Pasteurellaceae* pathogens (also corresponding to conducting a maximum of two diagnostic protocols per animal), precision of prevalence estimates was poor, with 95% confidence intervals including over 50% of all possible prevalence values (i.e., parameter space). Precision of prevalence estimates was better when two or more diagnostic protocols were conducted per animal and at least one of the protocols included a PCR test; however 95% confidence intervals still always included at least 37% of all possible prevalence values (Figure 1.2). Naïve prevalence estimates were generally similar to prevalence estimates that accounted for detection probability when multiple protocols (either replicating a single protocol or conducting different protocols) were conducted per animal (Figure 1.2).

Mycoplasma ovipneumoniae. Among the sampling events where *M. ovipneumoniae* was detected, the differences between the naïve estimates of prevalence for *M. ovipneumoniae* and the estimates that accounted for detection probability were small relative to those differences for other pathogens : 0.17 when a single diagnostic protocol was conducted from each of 29 animals using the TSB protocol (Hilgard), 0.09 when an average of 1.4 diagnostic protocols were conducted per each of 16 animals using the TSB and qPCR protocols (Stillwater), and 0.01 when two diagnostic protocols were conducted per each of 16 animals by replicating the TSB protocol (Highlands; Figure 1.2). The corresponding 95% confidence interval for the prevalence estimate that accounted for detection probability was inestimable for the Hilgard population because the prevalence estimate was at a parameter boundary (estimated to be 1), and included 53% (0.17-0.70) and 41% (0.07-0.48) of all possible prevalence values for the Stillwater and Highlands populations, respectively.

Mannheimia haemolytica. Among the sampling events where *M. haemolytica* was detected, the naïve prevalence estimate for *M. haemolytica* and the prevalence estimate that accounted for detection probability were dissimilar (Δ=0.67) when a single diagnostic protocol was conducted per each of 29 animals using the TSB protocol (Hilgard), but less so (Δ=0.07 and Δ=0.09) when at least two diagnostic protocols were conducted per animal in the Highlands (TSB protocol conducted twice per each of 16 animals) and Perma-Paradise (average of 2.4 diagnostic protocols conducted per each of 29 animals) populations. The corresponding 95% confidence intervals for the prevalence estimates that accounted for detection probability were wide, including 99% (0.01-1),

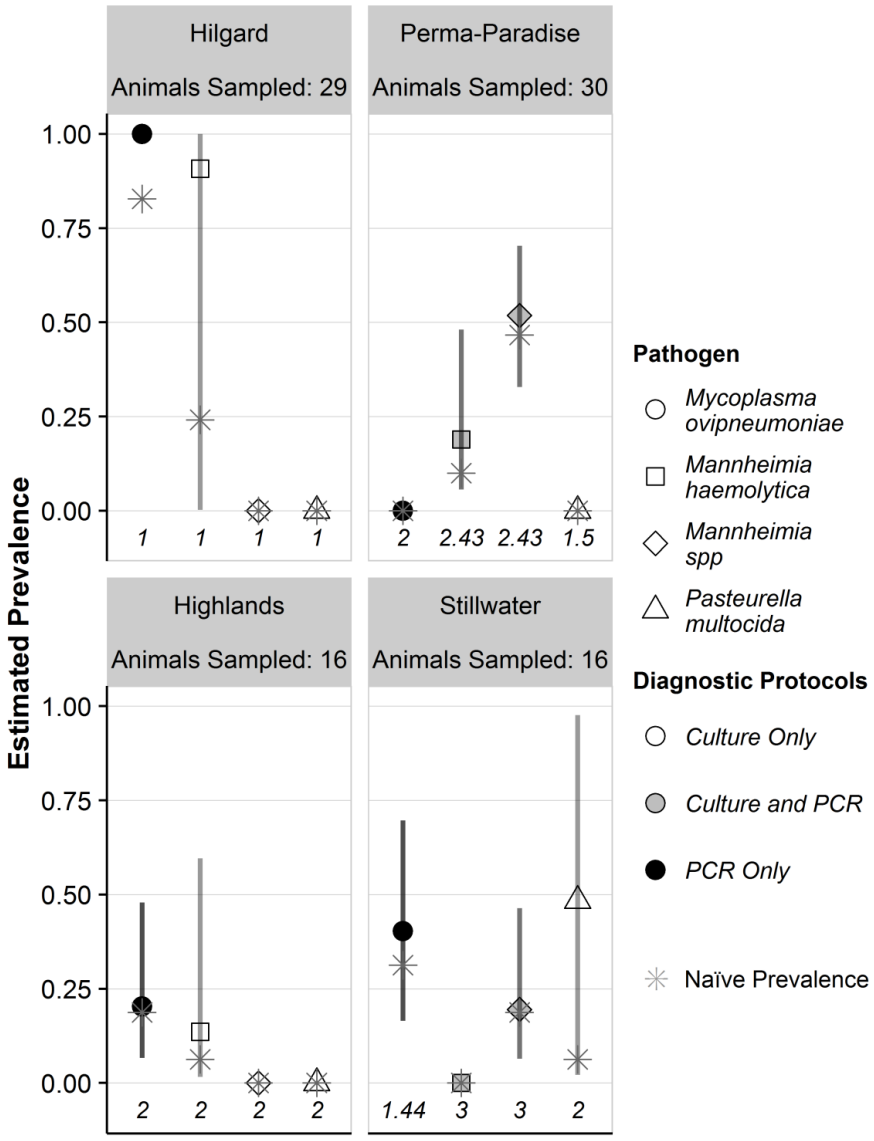


Figure 1.2. Estimated prevalence and 95% confidence intervals for four respiratory pathogens in four bighorn sheep populations. 95% confidence intervals were inestimable when detection probability was estimated at 0 or 1. Prevalence estimates obtained where protocols only used culture tests to detect pathogens are indicated by open symbols, those obtained where protocols used a combination of culture and PCR tests are shown as gray symbols and those obtained where only PCR tests were used are shown by black symbols. The mean numbers of protocols conducted per animal are shown across the x-axis of each panel. Naïve prevalence estimates (the proportion of animals a pathogen was detected in for a given sampling event) are indicated with gray asterisks. Prevalence estimates for *Mannheimia haemolytica* or *Mannheimia* spp. are for beta hemolytic or leukotoxigenic strains.

58% (0.02-0.60), and 42% (0.06-0.48) of all possible prevalence values for the Hilgard, Highlands, and Perma-Paradise populations, respectively.

Mannheimia species. *Mannheimia* spp. was detected in the Stillwater and Perma-Paradise sampling events (16 and 30 animals sampled , respectively; Figure 1.2). The naïve prevalence estimates for the Stillwater and Perma-Paradise populations were both very similar to the prevalence estimates that accounted for detection probability (Δ=0.01 and Δ=0.05, respectively). However, the 95% confidence intervals for the prevalence estimate that accounted for detection probability were imprecise, including 40% (0.06-0.46) and 37% (0.33-0.70) of all possible prevalence values for the Stillwater and Perma-Paradise populations, respectively.

Pasteurella multocida. *P. multocida* was only detected in the Stillwater population, where its presence was assessed by conducting two diagnostic protocols per each of 16 animals (Figure 1.2). There was a large difference between the naïve prevalence estimate and the prevalence estimate that accounted for detection probability (Δ=0.42). The 95% confidence interval for the prevalence estimate that accounted for detection probability included 96% of all possible prevalence values (0.02-0.98).

Power to Detect Pathogens at the Population-Level

Differences among Pathogens Using TSB Protocol. There was a large amount of variability in the power to detect the respiratory pathogens using the TSB protocol, and

the pathogens clustered into three groups. Power to detect *M. ovipneumoniae* was greatest, followed by *B. trehalosi* and *M. haemolytica*, then by *Mannheimia* spp. and *P. multocida* (Figure 1.3A). Under the default conditions (i.e., diagnostic protocol: TSB, animals sampled: 25, number of times protocol was conducted per animal: 1, pathogen prevalence: 0.10, population size: 100), power to detect *M. ovipneumoniae* was estimated at 0.87, *B. trehalosi* 0.61; *M. haemolytica* 0.51; *P. multocida* 0.29, and *Mannheimia* spp. 0.27. Increasing the number of animals sampled from 25 to 50 led to achieving adequate power (>0.80) to detect *B. trehalosi*, but did not achieve adequate power to detect any other targeted *Pasteurellaceae* pathogen (Figure 1.3A).

Differences among Diagnostic Protocols. Diagnostic protocol affected the power to detect each pathogen and there was a strong difference between the power of the TSB protocol and the protocol with the most power to detect each pathogen (Figure 1.4). For example, under default conditions, the power to detect *B. trehalosi* and *P. multocida* using the TSB protocol was estimated to be 0.61 and 0.29, respectively. In contrast, the power to detect these pathogens under default conditions using the Wyoming protocol was estimated to be 0.94 and 0.91, respectively. Although the difference in detection probability between the TSB and the most powerful protocols for *M. haemolytica* was not as substantial as for the other *Pasteurellaceae* pathogens, there were still notable differences in detection power. Under default conditions, the estimated detection power using the TSB protocol was 0.51 compared to 0.71 using the Wyoming protocol. There was little difference in the power to detect *M. ovipneumoniae* between the TSB protocol

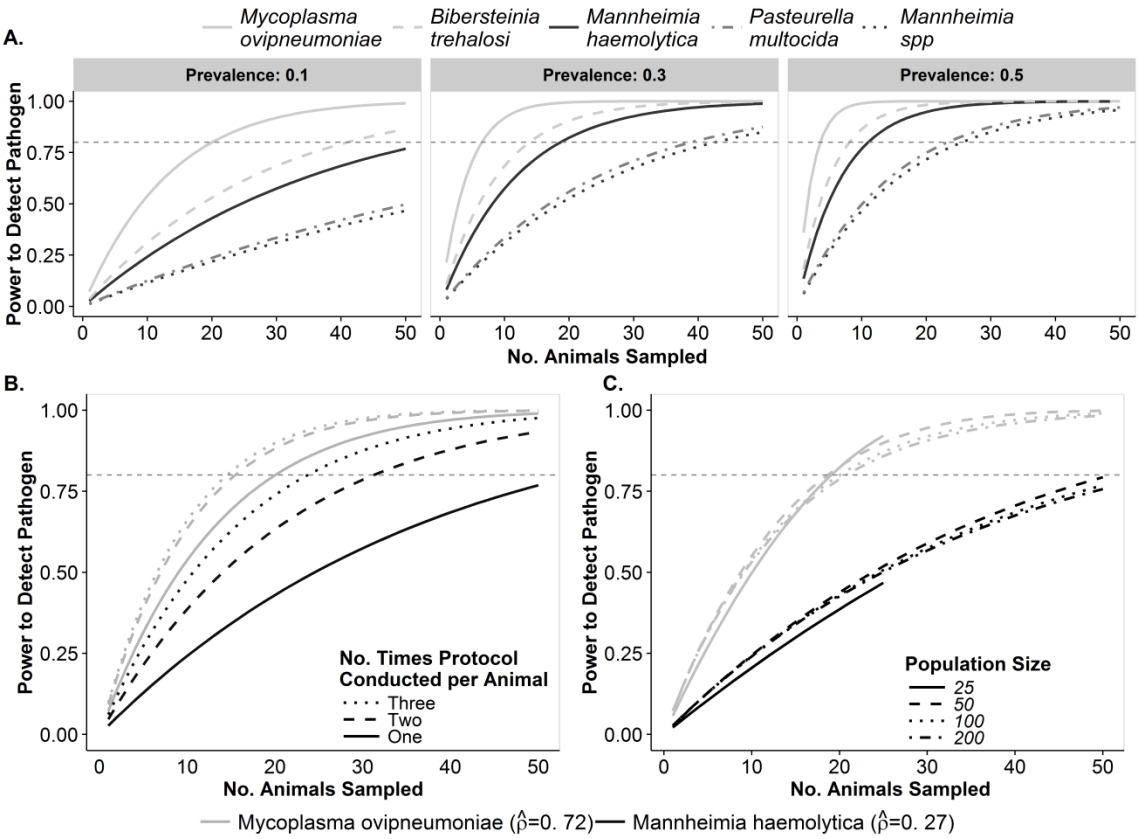


Figure 1.3. Power to detect five respiratory pathogens in bighorn sheep populations using the TSB protocols. Variability in power to detection pathogens at the population level is shown as it relates to different pathogens, population size, pathogen prevalence, number of animals sampled, and number of times protocols are conducted per animal. For all panels, each curve illustrates the power to detect each pathogen (y-axis) given the number of animals sampled from a population (x-axis). The horizontal dashed-gray line across each panel represents adequate (i.e., 80%) detection power. A. Variability in the power to detect each pathogen when the TSB protocol is conducted once per animal at three levels of pathogen prevalence in a population of 100. B. Effects of conducting TSB protocol multiple times per animal on power to detect two pathogens with either relatively high or relatively low detection probability in a population of 100 animals with 10% pathogen prevalence. C. Effect of population size on power to detect two pathogens with either relatively high or relatively low detection probability in a population of 100 animals with 10% pathogen prevalence.

and the Wyoming protocol and every protocol provided adequate power to detect *M.*

ovipneumoniae when the protocols were conducted once per each of 25 animals (Figure

1.4). Every *Pasteurellaceae* pathogen, except *Mannheimia* spp. could be reliably detected by conducting the Wyoming protocol once on each of 35 animals (Figure 1.4).

Sampling Effort. Increasing the number of animals sampled improved power to detect pathogens, but rates of improvement declined as the number of animals sampled increased (Figure 1.3A). For example, increasing the number of animals sampled for presence of *M. haemolytica* (under default conditions) from 15 to 25 increased the estimated detection power from 0.34 to 0.51 (Δ=0.17). Sampling 35 animals under default conditions increased the estimated detection power to 0.63(Δ=0.12). When 15 animals were sampled under default conditions, there was not adequate detection power for any pathogen and the estimated power to detect each of the *Pasteurellaceae* pathogens was less than 0.50. When 35 animals were sampled under otherwise default conditions, adequate power was achieved for *M. ovipneumoniae*, but not for any of the *Pasteurellaceae* pathogens (Figure 1.3A).

Detection power was markedly improved by conducting protocols multiple times per animal when detection probabilities were low. The largest gains occurred between conducting protocols one and two times per animal, and there were diminishing returns from conducting protocols a third time (Figure 1.3B). Under the default conditions described above where 25 animals were sampled, conducting the TSB protocol twice per animal increased estimated detection power of *M. haemolytica* from 0.51 to 0.72 (Δ=0.21) and conducting the TSB protocol three times per animal further increased estimated detection power to 0.82 (Δ=0.09). In contrast, power to detect *M. ovipneumoniae* under default conditions improved to 0.87, 0.94, and 0.95 when the TSB

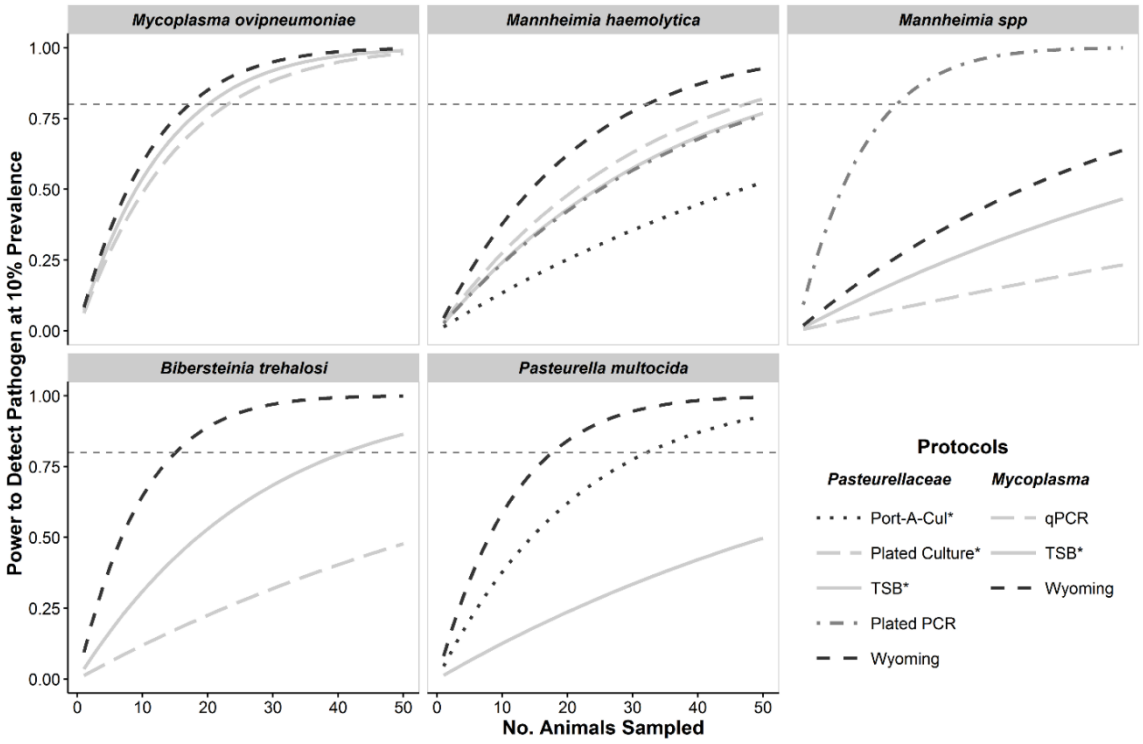


Figure 1.4. Power to detect five respiratory pathogens in bighorn sheep populations using different diagnostic protocols. For all panels, each curve illustrates the power to detect each pathogen at the population-level (y-axis) given the number of animals sampled from a population (x-axis) when the specified protocol was conducted once per animal. Within each panel, protocols where detection probability was estimated at zero or one are not shown. The horizontal dashed-gray line across each panel represents adequate (i.e., 80%) detection power.

protocol was conducted one, two, and three times per animal, respectively. When 35 animals were sampled and the TSB protocol was conducted multiple times per animal using, adequate detection power was also achieved for *M. haemolytica* (conducting protocol twice per animal), *B. trehalosi* (conducting protocol twice per animal), but not for *Mannheimia* spp. or *P. multocida* (Figure 1.5).

Pathogen Prevalence. Pathogen prevalence had a strong influence on the number of animals that would need to be sampled to achieve adequate detection power. For

example, adequate power (≥0.80) to detect *M. haemolytica* in a population at a prevalence of 0.50 could be attained by sampling just 11 animals under default conditions (Figure 1.3A). However, with a lower prevalence of 0.10, 55 animals would need to be sampled to attain adequate detection power (Figure 1.5). The effect of pathogen prevalence on necessary sampling effort was non-linear and most pronounced when prevalence was low. An increase in pathogen prevalence from 0.10 to 0.30 decreased the number of animals to be sampled to attain adequate power from 55 to 19 (Δ=36 animals). However, a further increase in prevalence from 0.30 to 0.50 only decreased the minimum number of animals to sample to 11 (Δ=8 animals, Figure 1.3A).

Population Size. Power to detect pathogens was minimally affected by population size (Figure 1.3C). Under default conditions, the estimated detection power for *M. haemolytica* was 0.47 in a population of 25 compared to 0.51 in a population of 100. Under default conditions for *M. ovipneumoniae*, detection power was 0.92 in a population of 25 and 0.87 in a population of 100. Small population size had the most notable effect on the power to detect pathogens simply due to the limited number of animals that could possibly be sampled (Figure 1.3C).

Discussion

These findings indicate that live-sampling of bighorn sheep for respiratory pathogens using diagnostic protocols that are readily available to most wildlife management agencies (i.e., available through an FFS laboratory) can lead to biased

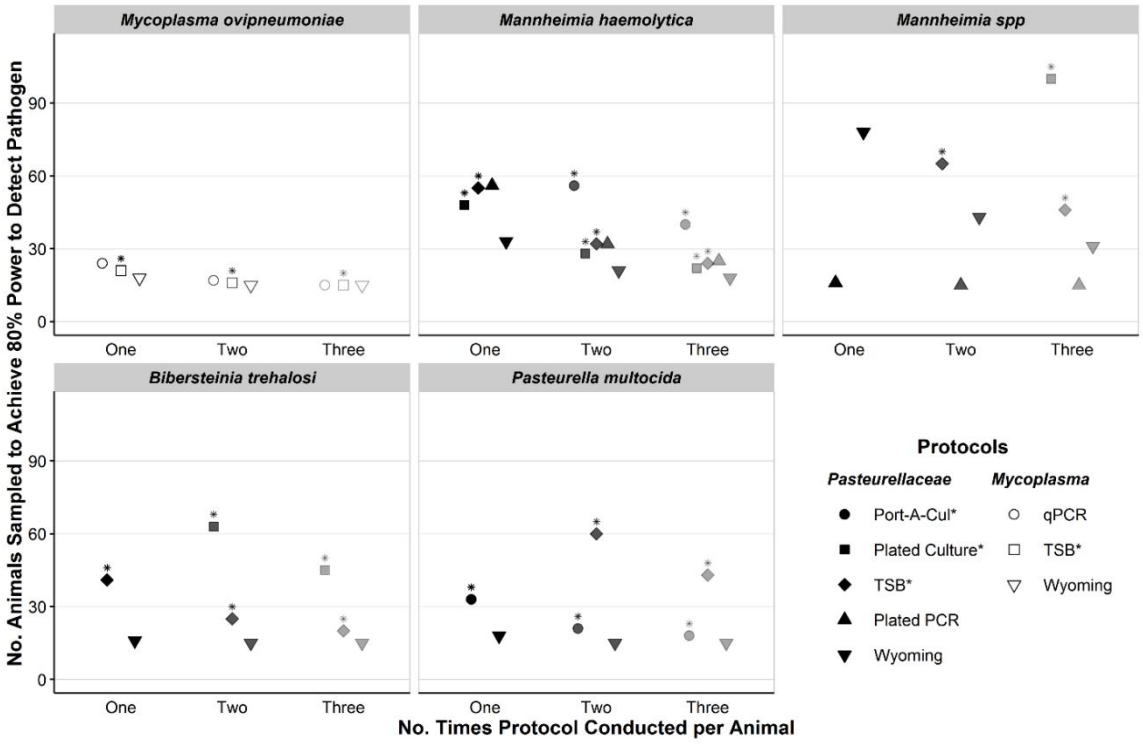


Figure 1.5. Minimum numbers of bighorn sheep to sample to achieve adequate detection power to detect respiratory pathogens. Minimum numbers to sample are those estimated to provide 80% power to detect a pathogen at 10% prevalence at the population-level using the specified protocol and number of samples per animal. One set of protocols was used to detect Pasteurellaceae organisms (shaded) and a separate set of protocols was used to detect *Mycoplasma ovipneumoniae* (not shaded). Protocols that used diagnostic tests offered by a fee-for-service laboratory are indicated with an asterisk (*) in the legend and above their symbols. Within each panel, protocols where detection probability was estimated at zero or one are not shown. The same is true for protocols where the estimated number of animals required to sample in order to attain adequate detection power was greater than 100.

assessments of respiratory pathogen communities. While the diagnostic test to detect *M. ovipneumoniae* offered by the FFS laboratory used in this study (WADDL) uses PCR with a high detection probability, only culture tests are offered by FFS laboratories to detect and identify *Pasteurellaceae* pathogens in bighorn sheep. Diagnostic protocols that relied solely on a FFS culture test for detection had low estimated detection probabilities (<0.50) for all *Pasteurellaceae* pathogens that were assessed. Low detection probability

of these protocols may be due in large part to diminished viability of targeted organisms during the process of delivery to the laboratory rather than sensitivity of the diagnostic test itself [16,26]. Nevertheless, this is a limitation whenever samples must be shipped to a laboratory for culture tests.

Low detection probability of *Pasteurellaceae* pathogens using FFS protocols makes simple assessment of species presence at the population-level unreliable when species are at low prevalence and populations are not intensively sampled. These conclusions generally corroborate those of the previous investigation of detection probability of *Pasteurellaceae* pathogens [14]. Although these specific findings apply to live-sampling bighorn sheep by swabbing the nasal cavity or tonsillar crypts, incongruent findings among studies investigating pathogen communities present in pneumonic and healthy lungs from the same respiratory disease epizootics [6,7] suggest that detection error affects these assessments as well. Thus, an assessment of detection probability applied to the sampling of lung tissues is warranted.

Naïve prevalence estimates of *Pasteurellaceae* pathogens are strongly biased when FFS diagnostic protocols are used, unless protocols are conducted multiple times per animal. Given poor detection power and biased prevalence estimates, any true associations between the presence of *Pasteurellaceae* organisms and historic or current respiratory disease in bighorn sheep would likely be unobservable using these protocols. The specificity issues that led to a generalized classification system for *Mannheimia* genus organisms further limit the ability to understand what role *Pasteurellaceae* pathogens play in bighorn sheep respiratory disease. In contrast to the *Pasteurellaceae*

pathogens, high detection probability for *M. ovipneumoniae* likely leads to more consistent detection and less biased naïve prevalence estimates in bighorn sheep populations where it is hosted.

These findings suggest that prevalence of any pathogen is estimated with poor precision unless intensive sampling is employed (i.e., many animals are sampled and protocols are conducted multiple times per animal), matching general expectations when detection error occurs [27]. Although *M. ovipneumoniae* could be reliably detected in a population by conducting a single protocol on a modest number of animals, its prevalence was estimated with low precision unless more sampling effort was invested. Therefore, variability in observed pathogen prevalence among different populations or different years within a population could be explained by either sampling variation or true variation in prevalence. Without accounting for differences in detection probability and sampling effort, differences in true prevalence remain unknown.

A simple and relatively inexpensive measure that wildlife management agencies can take to improve their ability to accurately characterize respiratory pathogen communities is to collect and assess two or three tonsil swabs from each live-sampled animal for *Pasteurellaceae* pathogens using FFS diagnostic protocols. Conducting protocols multiple times per animal would also provide agencies the ability to assess detection probability of their specific diagnostic protocols. These results suggest that 30 to 35 animals need to be sampled from a bighorn sheep population to reliably assess (>80% power) presence of *Pasteurellaceae* pathogens and *M. ovipneumoniae.* Either sampling 35 animals and conducting the *Pasteurellaceae* TSB protocol twice per animal

or sampling 30 animals and conducting the *Pasteurellaceae* TSB protocol three times per animal is predicted to result in adequate power to detect *M. haemolytica* and leukotoxigenic *B. trehalosi* at 10% prevalence. In either of these scenarios, *M. ovipneumoniae* can be reliably detected by conducting the TSB protocol just once per animal.

At least one protocol was estimated to have high detection probability for most *Pasteurellaceae* pathogens; however, detection probability for *M. haemolytica* was not significantly improved with any protocol. These results predict that 32 animals must be sampled using the most powerful protocol (Wyoming Protocol) to achieve adequate detection power. The improvement in estimated detection probability for *M. haemolytica* using the Wyoming protocol compared to the TSB or Plated PCR protocols corresponds closely to what would be expected by collecting two swabs, suggesting that the improvement may simply be the result of collecting two samples per animal. Evidence of multiple detection probabilities for leukotoxigenic *Mannheimia* spp. pathogens indicates that more specific classification measures are needed to provide sampling recommendations for this class of pathogens. Additionally, there is some evidence to suggest that *P. multocida* may be more readily detected in nasal swabs than tonsil swabs as a higher percentage of nasal swabs in the dataset that were assessed using the *Pasteurellaceae* TSB protocol tested positive for this pathogen.

Reliable detection of pathogens at the population-level, as defined in this study, still results in a 20% chance of a Type II error (false negative) for each pathogen. The power to simultaneously detect multiple pathogen species or strains in a population is

further reduced to the product of the power to detect each individually. Thus, the full set of respiratory pathogens hosted by free-ranging bighorn sheep populations will likely never be characterized with certainty. However, quantification of this uncertainty is possible using the concepts presented here and would lead to more accurate inference and informed management decisions. These specific findings may not be applicable to the diagnostic protocols used by all agencies that sample bighorn sheep for respiratory pathogens. However, these concepts can be applied to other diagnostic protocols (for which detection probability has been estimated) by using free and user-friendly online software (http://epitools.ausvet.com.au [28]), and manually calculating the per-animal detection rate if protocols are conducted multiple times per animal.

Poor detection power associated with FFS diagnostic protocols combined with hundreds of bighorn sheep translocations across North America suggest it is probable that *Pasteurellaceae* have been unknowingly introduced to new regions and populations. Suspected poor detection probability for *M. ovipneumoniae* prior to development of FFS PCR and serology tests [29], likely also resulted in the unknown introduction of this pathogen to new regions or host populations. Typically, bighorn sheep populations chosen to be source populations for translocations are those experiencing population growth, and thus, not exhibiting noticeable symptoms of respiratory disease. However, such populations may still host respiratory pathogens capable of causing disease [4]. Bighorn sheep source populations should be thoroughly sampled for respiratory pathogens using appropriate diagnostic protocols and sampling intensities to determine the extent to which these respiratory pathogens are present.

Resident respiratory pathogens within bighorn sheep populations exhibiting satisfactory demographic performance may pose a risk of future all-age respiratory disease epizootics within those populations [4]. Given poor power to detect respiratory pathogens at low prevalence using FFS live-sampling diagnostic protocols, initial detection of pathogens in bighorn sheep populations following observed respiratory disease may reflect an increase in prevalence or detectability [27,30] rather than an introduction of the pathogen to the population. Thus, taking measures to rigorously assess pathogen presence in populations with and without obvious signs of respiratory disease can provide multiple benefits for bighorn sheep conservation including informing translocation decisions, providing evidence for different sources of respiratory disease epizootics (i.e., novel vs resident pathogens) and elucidating additional measures that may be possible to prevent or mitigate respiratory disease in bighorn sheep.

Conclusions

Rigorous assessment of bighorn sheep respiratory pathogen communities is important for wildlife management agencies to inform potential translocations and to contribute to the understanding of respiratory disease in bighorn sheep. If adequate detection power for respiratory pathogens is not regularly achieved when bighorn sheep populations are sampled, test results have limited utility and are potentially misleading. These findings suggest that it is inadvisable to make strong inferences regarding causative agents of respiratory disease based on comparative estimates of pathogen prevalence unless resources are invested to accurately characterize pathogen communities. Relative to the total cost of sampling bighorn sheep, additional expenses to

improve characterization of respiratory pathogen communities are rather modest. Improved and consistent characterization of respiratory pathogen communities across numerous populations will help clarify the predominant causes of respiratory disease epizootics. The roles of the suspected respiratory pathogens, the predominant proximate causes of respiratory disease epizootics, and factors that could promote bighorn sheep populations to maintain vigor despite presence of respiratory pathogens are topics that may require multi-agency coordination and focused efforts to address. The information generated by this study may be used to inform future respiratory pathogen sampling efforts and allow rigorous comparison of pathogen communities assessed by different agencies and diagnostic protocols.

INCORPORATING UNCERTAINTY IN DETECTED RESPIRATORY PATHOGEN COMMUNITIES WHILE ASSESSING VARIATION IN DEMOGRAPHIC PERFORMANCE OF BIGHORN SHEEP POPULATIONS

Introduction

Most large-mammal populations across western North America were decimated following settlement by Europeans [31], though restoration efforts since the early 20th Century have been particularly successful in reestablishing ungulates in areas where suitable habitat remains [31]. However, while management actions have increased bighorn sheep (*Ovis canadensis*) numbers and distribution, this restoration has not achieved success comparable to most other ungulates, as bighorn sheep exist at less than 10% of historic population estimates [32] and often in small, isolated populations [3] that are more susceptible to a variety of potential threats including apparent competition [33,34], inbreeding depression [35], along with a variety of factors that collectively amount to the Allee effect [36,37]. Additionally, respiratory disease (pneumonia) is a primary factor that has hindered bighorn sheep restoration and plays a primary role in dictating management policies for the species [38].

Respiratory disease epizootics are typically first expressed as all-age mortality events, and are followed by multiple years of depressed juvenile survival [2], though mortality rates and duration of depressed lamb survival varies. Ultimate outcomes following initial respiratory disease epizootics in bighorn sheep populations have ranged from full recovery to local extinction or depopulation [12]. Evidence strongly indicates that domestic sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*) typically host

respiratory pathogens which cause this disease in bighorn sheep [39,40]. Accordingly, maintenance of separation between domestic sheep or goats and wild sheep is the primary management tool wildlife agencies use to minimize the occurrence of respiratory disease outbreaks [38].

Respiratory disease in bighorn sheep has been described as a poly-microbial disease [1] and multiple pathogens have been associated with the disease. Leukotoxigenic members of the *Pasteurellaceae* family, particularly *Mannheimia haemolytica* (*M. haemolytica*), are regularly detected in the lungs of bighorn sheep that have succumbed to respiratory disease [7,41,42] and direct inoculation of leukotoxigenic *M. haemolytica* or *Bibersteinia trehalosi* (*B. trehalosi*) leads to fatal pneumonia in bighorn sheep, but not domestic sheep (*Ovis aries*). *Pasteurella multocida* (*P. multocida*), though not leukotoxigenic, has also been associated with respiratory disease epizootics in free-ranging bighorn sheep populations [6]. *Mycoplasma ovipneumoniae (M. ovipneumoniae)* has been associated with respiratory disease in free-ranging bighorn sheep populations [6,29] and experimental introduction has resulted in epizootic respiratory disease in bighorn sheep, but not in co-penned domestic sheep [8]. Additionally, commingling of bighorn sheep with domestic sheep without evidence of exposure to *M. ovipneumoniae* resulted in a lower mortality rate for bighorn sheep than had previously been observed in similar studies [43]. The reported roles of *Pasteurellaceae* pathogens and *M. ovipneumoniae* relative to each other have ranged from non-necessary facilitation of pasteurellosis by *M. ovipneumoniae* [44] to *M. ovipneumoniae*-caused ciliostasis leading

to secondary bacterial infections by *Pasteurellaceae,* among numerous other commensal bacteria [1].

Evidence that either *M. ovipneumoniae* or *Pasteurellaceae* pathogens cause respiratory disease in bighorn sheep is largely based on the observed responses of naïve animals to the introduction of these pathogens. Thus, most management efforts have attempted to reduce respiratory disease epizootics in wild bighorn sheep by maintaining separation with domestic caprids and, thereby, preventing the introduction of novel pathogens. However there is evidence to suggest that introduction of novel pathogens may not explain all bighorn sheep respiratory disease events as; a) bighorn sheep populations have long been exposed to domestic sheep and their exotic pathogens [45]; b) over 150 respiratory disease epizootics have been documented in bighorn populations over the last 30 years (Western Association of Fish & Wildlife Agencies, unpublished data) despite significant reductions in domestic sheep throughout bighorn sheep range; and c) there are robust bighorn sheep populations known to be exposed to each of the pathogenic agents linked to bighorn sheep respiratory disease [1,46]. Pathogens introduced into host populations are expected to remain circulating unless the host population size drops below a critical community size; however, this threshold is rarely known in wildlife populations [47] and lack of disease expression does not necessarily indicate a pathogen has become extinct from a host population. Increases in pathogen virulence, transmission rates, or the accumulation of susceptible individuals (e.g., via recruitment of naïve young) may then result in resident pathogens causing periodic disease events [4,5,48]. Thus, some portion of respiratory disease epizootics may be

caused by resident pathogens that have existed in bighorn sheep populations for years without causing overt signs of disease [5] and demographically robust populations with no recent history of disease may be reservoirs for pathogenic agents. This possibility has important implications for management of bighorn sheep respiratory disease in that the presence of hypothesized respiratory pathogens in populations with strong demographic rates may pose a risk to the host populations as well as adjacent populations [4,49], but would also suggest that such populations may possess traits that provide increased resilience to respiratory disease.

Specific pathogens have been linked to respiratory disease and the effects of documented respiratory disease on demographic rates, such as recruitment, adult survival, and population growth have been described [50,2,51,52]; however variability in demographic rates in bighorn sheep populations, with respect to the presence of *Pasteurellaceae* or *M. ovipneumoniae* has not been well described. Uncertainties related to roles these pathogens play in causing respiratory disease and to what extent demographically robust populations host these pathogens could be addressed by such an assessment. If the suspected respiratory pathogens are detected in numerous bighorn sheep populations with strong demographic performance, potential mechanisms that reduce the demographic effects of respiratory pathogens may be elucidated. Such understanding could be used to develop management strategies that adopt a modern definition of wildlife health, promoting resilience of bighorn sheep populations to the numerous challenges, including respiratory disease, that they face on the modern landscape [53]. Additionally, rigorous documentation of the presence of respiratory

pathogens across many bighorn sheep populations can also inform the feasibility of different management options depending on the pervasiveness of the pathogens.

Here, coordinated efforts were used across Montana and Wyoming to rigorously assess respiratory pathogen communities in a diverse set of bighorn sheep populations and then relate estimates of average recruitment and population growth to presence of *Pasteurellaceae* and *M. ovipneumoniae*. The primary objectives of this study were to: 1) assess the pervasiveness of the hypothesized respiratory pathogens in the study populations; 2) assess whether presence of any specific pathogen or combination of pathogens is associated with differences in recruitment or population growth; and 3); determine the extent to which populations hosting different suspected respiratory pathogens maintained satisfactory recruitment or population growth rates. Little or no association between demographic performance and presence of suspected respiratory pathogens were hypothesized and, given the long history of domestic sheep grazing in the two states [45], it was hypothesized that the suspected respiratory pathogens are hosted by the majority of study populations.

Methods

Study Populations

This study included 17 bighorn sheep populations that occupy a wide range of habitat types across Montana and Wyoming and have varying disease and management histories (Table 2.1). Populations were defined according to how agency personnel conduct and report annual population surveys and typically corresponded to hunting

districts defined by the respective state wildlife management agencies. The areas inhabited by these populations represent much of the variation in habitat types that are realized across the species' range. Topography in the ranges of the study populations varies from rugged badlands areas, with 300 meters of vertical relief to large mountain ranges with over 2,000 meters of vertical relief. Annual precipitation in the ranges varies from 30 cm to over 100 cm. Inhabited ecological regions include northern Rocky Mountains, northern Rocky Mountain Foothills, northern rolling plains, and central Rocky Mountains [54]. Predator communities range from completely intact communities, with all historic predators present on the landscape including wolves (*Canis lupus*), coyotes (*Canis latrans*), grizzly bears (*Ursus arctos*), black bears (*Ursus americanus*), wolverines (*Gulo gulo*), golden eagles (*Aquila chrysaetos*), and mountain lion (*Puma concolor*), to less diverse predator communities containing golden eagles, mountain lions, black bears, or coyotes. Study populations primarily occupy public lands but seasonally use areas of private land.

Study populations represent completely native populations (n=10), completely restored populations (n=6), as well as native populations that have been augmented in efforts to increase population size (n=1). Connectivity level varies among study populations, which include well-connected metapopulations (n=10), populations thought to have limited connectivity (n=2), and populations thought to be mostly isolated (n=5). Population structure, in terms of number of subpopulations, ranged from one sub-population to over ten sub-populations. Respiratory disease histories in the populations include no documented history of disease (n=8), a single all-age epizootic (n=4) and

multiple all-age epizootics (*n*=5). Estimated population size for individual populations ranged from 75 to 1000 (Appendix F, Table F1). Rams were harvested from all study populations with mean annual ram harvest ranging from 1.2 to 55. Ewes are currently harvested from five study populations (Appendix F, Table F1) with mean annual ewe harvest of these populations ranging from three to 16. Density-reduction translocations have occurred in two study populations and augmentation translocations have occurred in two study populations since 2011 (Appendix F, Table F1).

Animal Capture and Sampling

A total of 637 individual bighorn sheep from 17 populations in Montana and Wyoming were captured and sampled between December and March of each year from 2012-2016. The number of animals sampled from a single population ranged from 10 (Dubois Badlands) to 124 (Hilgard). Animals were captured using chemical immobilization, baited drop nets, or helicopter net-gunning and live-sampled for presence of *Mycoplasma ovipneumoniae,* leukotoxigenic *M. haemolytica or Mannhemia glucosida* (combined as *M. haemolytica* as these two species are not reliably differentiated by available diagnostic tests [7]), leukotoxigenic *Mannheimia ruminalis* or *Mannheimia* spp. (combined as *Mannheimia* spp. because the ability to identify *Mannheimia ruminalis* from other species was not available until the final year of data collection), leukotoxigenic *B. trehalosi,* and *P. multocida.* Presence of *Pasteurellaceae* pathogens was assessed by using a sterile polyester-tipped applicator (Puritan #25-806 1PD, Guilford, Maine, USA) to swab the tonsillar crypts (with the aid of a lighted swab

Table 2.1. General attributes of the 17 bighorn sheep populations investigated in this study

Population	Hunt-Area	All-age epizootics	Herd Origin	Sub-Pops[1]	Connectivity Level	Migratory	Ecological Region[2]	Elevation Range (m)
Perma-Paradise	MT-124	None	Restored	2	Isolated	No	Northern Rocky Mtn.	800-2000
Petty Creek	MT-203	None	Restored	2	Isolated	No	Northern Rocky Mtn.	1000-2200
Lost Creek	MT-213	1991,2010	Restored	2	Limited	Yes	Central Rocky Mtn.	1700-3200
Highlands[3]	MT-340	1995	Restored	3	Isolated	No	Central Rocky Mtn.	1500-3100
Castle Reef	MT-422	1925, 1932, 1984, 2010	Native	3	Meta Population	Yes	Central Rocky Mtn./ N. Rocky Mtn. Fthls	1400-2900
Fergus	MT-482	None	Restored	4	Meta Population	No	Northern Rolling Plains	700-900
Middle Missouri Breaks	MT-622	None	Restored	3	Isolated	No	Northern Rolling Plains	700-1000
Hilgard	MT-302	1997	Augmented -Native	3[4]	Isolated	Yes	Central Rocky Mtn.	2100-3400
Upper Yellowstone	MT-999[5]	2012, 2014	Native	8	Meta Population	Yes	Central Rocky Mtn.	1500-3400
Stillwater	MT-500a	Pre-1920	Native	2	Limited	Yes	Central Rocky Mtn.	1500-3900
Clark's Fork	WY-1	None	Native	>10	Meta Population	Yes	Central Rocky Mtn.	1400-3600
Trout Peak	WY-2	None	Native	>10	Meta Population	Yes	Central Rocky Mtn.	1900-3800
Wapiti Ridge	WY-3	None	Native	>10	Meta Population	Yes	Central Rocky Mtn.	1900-3800
Franc's Peak	WY-5	2011-2013	Native	>10	Meta Population	Yes	Central Rocky Mtn.	2000-4000
Dubois Badlands	WY-22	None	Native	2	Meta Population	Yes	Central Rocky Mtn.	2200-3800
Whiskey Basin	WY-10	1991	Native	>5	Meta Population	Yes	Central Rocky Mtn.	2300-4200
Jackson	WY-7	2002, 2012	Native	6	Meta Population	Yes	Central Rocky Mtn.	2100-3600

[1] Sub-population defined as self-sustaining group of ewes occupying distinct winter range [55]
[2] Ecological region defined according to USDA Handbook 296 [54]
[3.] Recent demographic data were not available for the Highlands populations
[4.] One sub-population was established from another sub-population in 2015
[5.] The Upper Yellowstone population is monitored as a single population but inhabits four hunting districts.

extender and tongue depressor) and, for a subset of animals, the nasal cavity. Presence of *M. ovipneumoniae* was assessed by using polyester-tipped applicators to swab the nasal cavity. Five diagnostic protocols were used to detect *Pasteurellaceae* pathogens and three were used to detect *M. ovipneumoniae.* Complete descriptions and estimates of detection probability (i.e., sensitivity) for each protocol were estimated in Chapter 1 (Appendix A). Additionally, lung samples from 14 bighorn sheep that died in an all-age respiratory disease epizootic in the Upper Yellowstone Complex were collected by trained personnel and tested for *Pasteurellaceae* and *M. ovipneumoniae.*

Pasteurellaceae Protocols. Three of the five *Pasteurellaceae* protocols entailed shipping samples in transport media to a fee-for-service (FFS) laboratory (Washington Animal Disease Diagnostic Laboratory-WADDL) for bacterial identification via culture diagnostic tests. Bacterial identification for the remaining two *Pasteurellaceae* protocols was conducted at a non-FFS laboratory (Wyoming Game and Fish Department Diagnostic Laboratory-WGFD) using culture and/or polymerase chain reaction (PCR) diagnostic tests. Of the three *Pasteurellaceae* protocols where diagnostic tests were conducted at WADDL, one protocol entailed freezing samples in tryptic soy broth with 15% glycerol (TSB; Hardy Diagnostics, Santa Maria, California, USA) and shipping overnight on dry ice (TSB protocol); one protocol entailed immediate inoculation and incubation of a Columbia Blood Agar (CBA; Hardy Diagnostics, Santa Maria, USA) plate, swabbing the primary streak zone of the CBA plate, and overnight-shipping the swab sample in TSB on dry ice (Plated Culture protocol); and one protocol entailed placing samples cool in Port-A-Cul™ transport media tubes (BD, Sparks, Maryland,

USA) and shipping overnight on ice packs (Port-A-Cul protocol). Of the two *Pasteurellaceae* protocols where bacterial identification was performed at WGFD, one protocol entailed collecting two tonsil swab samples per animal, using the first to immediately inoculate and incubate a CBA plate, placing the second sample in a Port-A-Cul™ transport media tube for approximately six hours before inoculating and incubating a second CBA plate, and then using a combination of culture and PCR tests to identify bacteria on the plates (Wyoming protocol). The other protocol entailed using samples to immediately inoculate CBA plates and then incubating the plates for 48 hours before washing all bacterial growth from the plate and freezing in phosphate buffered saline (PBS) until PCR tests were conducted at WGFD (Plated PCR protocol).

Mycoplasma ovipneumoniae Protocols. Three diagnostic protocols were used to detect *M. ovipneumoniae;* one protocol entailed freezing samples in TSB and overnight-shipping on dry ice to WADDL for an FFS PCR test (TSB protocol); one protocol entailed placing samples in empty sterile vials and shipping overnight on dry ice for a non-FFS PCR test (qPCR protocol); and one protocol entailed placing samples cool in Port-A-Cul™ transport media tubes or Amies media without charcoal for up to four hours before transferring the sample to modified tryptone soy broth (TSB-1) and incubating for 48 hours prior to conducting PCR tests at WGFD. Exposure of individual bighorn sheep to *M. ovipneumoniae* was also assessed by submitting 1mL of serum to WADDL for an FFS serology test.

Lung Tissue Protocol. Lungs were collected from bighorn sheep and delivered to the Montana Department of Fish, Wildlife and Parks Wildlife Health Lab where the lungs were grossly inspected by trained personnel, who collected tissue samples and submitted them on dry ice to WADDL for *Pasteurellaceae* culture and *M. ovipneumoniae* PCR.

Demographic and Population Characteristic Data Collection

Demographic data used in this analysis were primarily collected by Montana Department of Fish Wildlife and Parks (FWP) or Wyoming Game and Fish Department (WGFD) personnel as part of regular bighorn sheep population surveys from 2006 to 2016. Population surveys were typically conducted January-February or March-April on an annual basis and data collected were used to index trends in population abundance (trend-counts) as well as estimate age and sex ratios (classification counts). For one study population (Fergus), trend-counts were conducted during the summer (July-August) and classification counts were collected during the spring (April-May). For three study populations (Clark's Fork, Trout Peak, Wapiti Ridge) winter classification counts, but not trend counts, were conducted annually. The majority of population surveys were conducted from an aerial platform, though surveys for several populations occupying readily available winter ranges were conducted entirely or partly from the ground (e.g., Castle Reef, Hilgard, Lost Creek, and Stillwater). For each population, personnel counted and classified all observed individuals within a consistent area of core winter range across years. If populations were surveyed multiple times within a winter season, the maximum number of animals within each age and sex class that were observed across

surveys were recorded as the counts for that year. Personnel overseeing each population were queried to identify and censor surveys where accuracy was suspected to be inadequate. These personnel were also queried to provide five and ten-year population trends (decline, stable, increase), recent population estimates, population objective, history of respiratory disease, population structure (i.e., number of distinct sub-populations within the population, defined following Festa-Bianchet [55]), and connectivity level with other bighorn sheep populations (isolated, limited, metapopulation) based on their professional judgment.

Indices of Demographic Performance

Demographic performance of study populations was characterized by their mean recruitment rates and geometric population growth (lambda or $\bar{\lambda}$). Recruitment rates were indexed as the ratio of lambs to adult females (lamb: ewe ratio) that were counted in the classification surveys. Mean geometric population growth was indexed using counts from annual surveys after accounting for purposeful management additions or removals (described in detail in *Population Growth and Pathogen Detection* section of methods). Mean annual population growth ($\bar{\lambda}$) was considered satisfactory if greater than 1 and average recruitment was considered satisfactory if lamb:ewe ratios were greater than 0.20 [11]. Populations that experienced all-age epizootics within the range of years that recruitment data were collected were split into separate populations (before epizootic and after epizootic) for analyses if pathogen data were collected before and after epizootics: If pathogen data were not collected before epizootics, demographic data preceding the die-

off were excluded from analysis. The Highlands population was not considered in demographic analyses as recent data were not available.

Estimating Power to Detect Pathogens

If a pathogen was detected in a study population, it was assumed that the pathogen was present each year the population was sampled, unless an all-age respiratory disease event occurred during the study. For populations where a specific pathogen was never detected, the probability that the employed sampling methodology would detect the pathogen (i.e., detection power) at low prevalence (10%) in each population was estimated as a function of the estimated size of the population (shown in Appendix F, Table B1), the estimated pathogen-specific detection probabilities of the protocols that were employed (Appendix E, Table E3), the number of times each protocol was conducted per animal (Appendix E; Table E1, Table E2), and the number of animals that were tested using each protocol (Appendix E; Table E1, Table E2). Complete derivation of the protocol-specific detection power equation is shown in Appendix A. Detection probability was inestimable for several pathogen-protocol combinations, presenting a challenge for estimating detection power when these protocols were employed. The issue was addressed separately depending on the reason the parameter was inestimable and explained in detail in Appendix E.

Where multiple protocols were used to assess pathogen presence in a population (*t*), overall detection power for that population was estimated by grouping sampled animals into cohorts with identical combinations of protocols used to test them for respiratory pathogens. The equation for per-animal probability of not detecting a

pathogen, $\Pr(T^-)$, that was derived in Appendix C was expanded to accommodate multiple protocols conducted per animal. If a single protocol is considered: $\Pr(T^-)\ (1 - \alpha/(\alpha + \beta))^s$ (as in Appendix C), where α and β describe the detection probability distribution for the employed protocol and s represents the number of replicate trials conducted per animal. The per-animal probability of not detecting a pathogen for each cohort, $\Pr(T^-)_c$, was calculated as the product of the per-animal probabilities of not detecting a pathogen for each individual protocol (p):

$$\Pr(T^-)_c = \prod_{p=1}^{\#\ protocols} (1 - a_p/(a_p + \beta_p))^{S_p}$$

The modified per-animal probability of not detecting a pathogen for each cohort was then scaled to the per-cohort probability of not detecting a pathogen, $\Pr(N^-)_c$, following the approach shown in Appendix C. Overall detection power across all cohorts of sampled animals in a population was calculated as the complement of the product of the per-cohort probabilities of not detecting a pathogen:

$$\Pr(N^+) = 1 - \prod_{c=1}^{\#\ cohorts} \Pr(T^-)_c$$

Where pathogen sampling occurred over multiple years and a pathogen was never detected, it was assumed that population size did not change to a significant degree and that the pathogen was present through the entire duration of sampling, allowing sampling across multiple years to be treated as a single sampling event.

Recruitment and Pathogen
Detection Analysis

The most recent five years of recruitment data for each study population were included in recruitment analysis, with exception of populations where all-age respiratory epizootics have been documented since 2011. In these cases the censoring procedure described previously was applied. The recruitment dataset was restricted to the most recent five years to minimize the extrapolation of recent pathogen data to historic recruitment rates. It was assumed that the pathogen community detected in each study population did not change during the time-series as the introduction of influential pathogens is expected to result in all-age disease epizootics. To explore the possibility that influential pathogens were introduced to or went extinct from study populations during the time series, a piecewise regression analysis was conducted for each study population. The piecewise regression attempted to identify if and when recruitment rates fundamentally changed, indicating a potential change in pathogen community (see Appendix F for details).

Differences in mean recruitment rates with respect to the detection or non-detection of different respiratory pathogens in the study populations were assessed using a Poisson random-intercept model run using the "lme4" package [56] in Program R. The following model was used for each respiratory pathogen, using a random intercept for study populations and a binary fixed-effect variable indicating whether the pathogen was detected in each population:

$$\mu_j = \beta_0 + \beta_1 \cdot Detected_j + b_j,$$

where μ_j is the mean $log\left(\frac{lambs}{ewes}\right)$ for population j and b_j is the random intercept of population. The limited sample of study populations precluded assessing effects of multiple pathogens in the same model. To minimize the probability of falsely considering a pathogen absent from a population in the analysis, populations were censored from analysis where a specified pathogen was not detected and there was not adequate detection power (>80%) at 10% prevalence.

Population Growth and Pathogen Detection Analysis

The technique described by Eberhardt was used to estimate average annual population growth after accounting for harvest and translocations [57,58] because several populations have been intensively managed to reduce population size. Most populations were surveyed between annual management removals/additions and the lambing season. Annual estimates of population growth (lambda or λ) for these populations were obtained assuming population growth occurred before the most recent management removals/additions [57]. Annual trend surveys for one population (Fergus) were conducted after lambing and before management removals/additions. Lambda for this populations was estimated assuming population growth occurred after the most recent management removals/additions [57]. When trend counts were not conducted in a single year, average lambda over the two-year interval between the previous and successive years' counts was estimated using polynomial equations [57]. Lambda was not estimated across intervals where trend counts were not conducted in multiple consecutive years. Population growth of the study populations was assessed assuming three different

sighting probabilities that have been reported for bighorn sheep (50%, 85%) [52,59] or observed in these study populations (70%, Montana State University, *unpublished data*). Due to suspected high levels of variability in detection error across years, average lambda was not assessed for populations where less than four annual estimates of lambda were available to minimize potential effects of sampling error on overall conclusions. This resulted in censoring four study populations from this analysis (Upper Yellowstone Post-2013, Clark's Fork, Trout Peak, and Wapiti Ridge).

The geometric mean and standard error of the annual lambda (λ) estimates were calculated to estimate the overall average lambda for each study population. The one-sided confidence level that the geometric mean lambda ($\bar{\lambda}$) of each study population was greater than one was also calculated for each study population. This confidence-level was estimated using a one-tailed, one-sample t-test assessing evidence against a null hypothesis that the geometric mean lambda was greater than one. Differences in confidence levels that population growth was positive ($\bar{\lambda} > 1$) with respect to the detection or non-detection of different respiratory pathogens in the study populations were assessed using a two-tailed Mann-Whitney test for each pathogen. To minimize the probability of falsely considering a pathogen absent from a population in the analysis, populations were censored from analysis where a specified pathogen was not detected and there was not adequate power (>80%) to detect it at 10% prevalence.

Results

Pathogen Detection

The median number of respiratory pathogen species detected per study population (considering only the five species of interest described in this study) was three (3). None of the pathogen species of interest were detected in one study population (Middle Missouri Breaks), two pathogen species were detected in three study populations (Perma-Paradise, Petty Creek, Lost Creek), three pathogen species were detected in four study populations (Castle Reef, Fergus, Upper Yellowstone, Dubois Badlands), four pathogen species were detected in one study population (Stillwater), and all five pathogen species were detected in seven study populations (Hilgard, Clark's Fork, Trout Peak, Wapiti Ridge, Franc's Peak, Whiskey Basin, Jackson). Collectively, leukotoxigenic *Pasteurellaceae* were detected in every study population except Middle Missouri Breaks. *M. ovipneumoniae* was detected in all populations where leukotoxigenic *Pasteurellaceae* were detected, while leukotoxigenic *Pasteurellaceae* were detected in two populations (Perma-Paradise, Petty Creek) where *M. ovipneumoniae* was not detected (Figure 2.1). Out of the 6820 individual bighorn sheep estimated to exist in the 17 study populations, 5935 (87%) live in populations known to carry *M. ovipneumoniae* and 6420 (94%) live in populations known to carry leukotoxigenic *Pasteurellaceae.*

Three of the five respiratory pathogens were detected in over 70% of the study populations. *M. ovipneumoniae* was detected in 14 of 17 (82%) study populations and was not detected in the Perma-Paradise, Petty Creek, or Middle Missouri Breaks populations (Figure 2.1). Leukotoxigenic *M. haemolytica* was detected in 13 of 17 (71%)

study populations and leukotoxigenic *Mannheimia* spp. was detected in 14 of 17 (82%) study populations. *P. multocida* and leukotoxigenic *B. trehalosi* were both detected in 9 of 17 (56%) study populations including all Wyoming study populations and two Montana study populations (Stillwater, Hilgard) that are adjacent to Wyoming. Presence of *M. haemolytica, Mannh*eimia *spp.,* or *P. multocida* was not assessed with 80% confidence in any population where they were not detected (Figure 2.1), preventing association of population characteristics with presence of these pathogens. Presence of *M. ovipneumoniae* was reliably assessed (>80% confidence) in all three populations where it was not detected and presence of *B. trehalosi* was reliably assessed in three (Perma-Paradise, Castle Reef, Fergus) of the eight populations where it was not detected (Figure 2.1).

Population Attributes and Pathogen Detection

Six of 17 (35%) study populations did not meet population the objective (i.e., estimated population size was less than 90% of objective population size for Montana populations or less than 80% of objective population size for Wyoming populations; Appendix F Table F1). Six of 16 populations (37.5%) were thought to have a declining ten-year population trend, four (25%) were thought to have a stable ten-year population trend, and six (37.5%) were thought to have an increasing ten-year population trend. Mean five-year lamb:ewe ratios were satisfactory (>0.20) for 13 of 16 (81%) populations where adequate demographic data were available and were less than 0.20 for Lost Creek, Castle Reef, and Dubois Badlands (Appendix F Table F1).

Most (≥50%) populations had satisfactory recruitment (five-year mean lamb:ewe ratio >0.20), and were thought to have stable or growing ten-year population trends regardless of whether or not any specific respiratory pathogen was or was not detected (Table 2.2). None of the three (0%) populations where *M. ovipneumoniae* was not detected had any documented history of respiratory disease and nine of the 14 (64%) populations where it was detected had a history of respiratory disease. There were no apparent difference in history of respiratory disease for populations where *B. trehalosi* was or was not detected (Table 2.2). The proportions of populations that met management objective, had adequate recruitment, or had stable or growing population trends were similar between populations where *M. ovipneumoniae* or *B. trehalosi* was and was not detected. All three populations where *M. ovipneumoniae* was not detected met management objective, had adequate recruitment rates, and had stable or growing population trends (Table 2.2).

Recruitment Rates and Pathogen Detection

The median five-year mean lamb:ewe ratio across the study populations was 0.30 and five-year mean lamb:ewe ratios of individual populations ranged from 0.09 (Castle Reef) to 0.48 (Middle Missouri Breaks). Mean five-year lamb:ewe ratios were between 0.20 and 0.30 for four populations (Fergus, Clark's Fork, Trout Peak, Wapiti Ridge), were between 0.30 and 0.40 for five populations (Perma-Paradise, Upper Yellowstone, Clark's Fork, Whiskey Basin, Jackson), and were 0.40 or greater for four populations (Petty Creek, Middle Missouri Breaks, Hilgard, Stillwater; Appendix F Table F1). Piecewise regression analysis identified break-years for 12 of the 16 study populations, before and

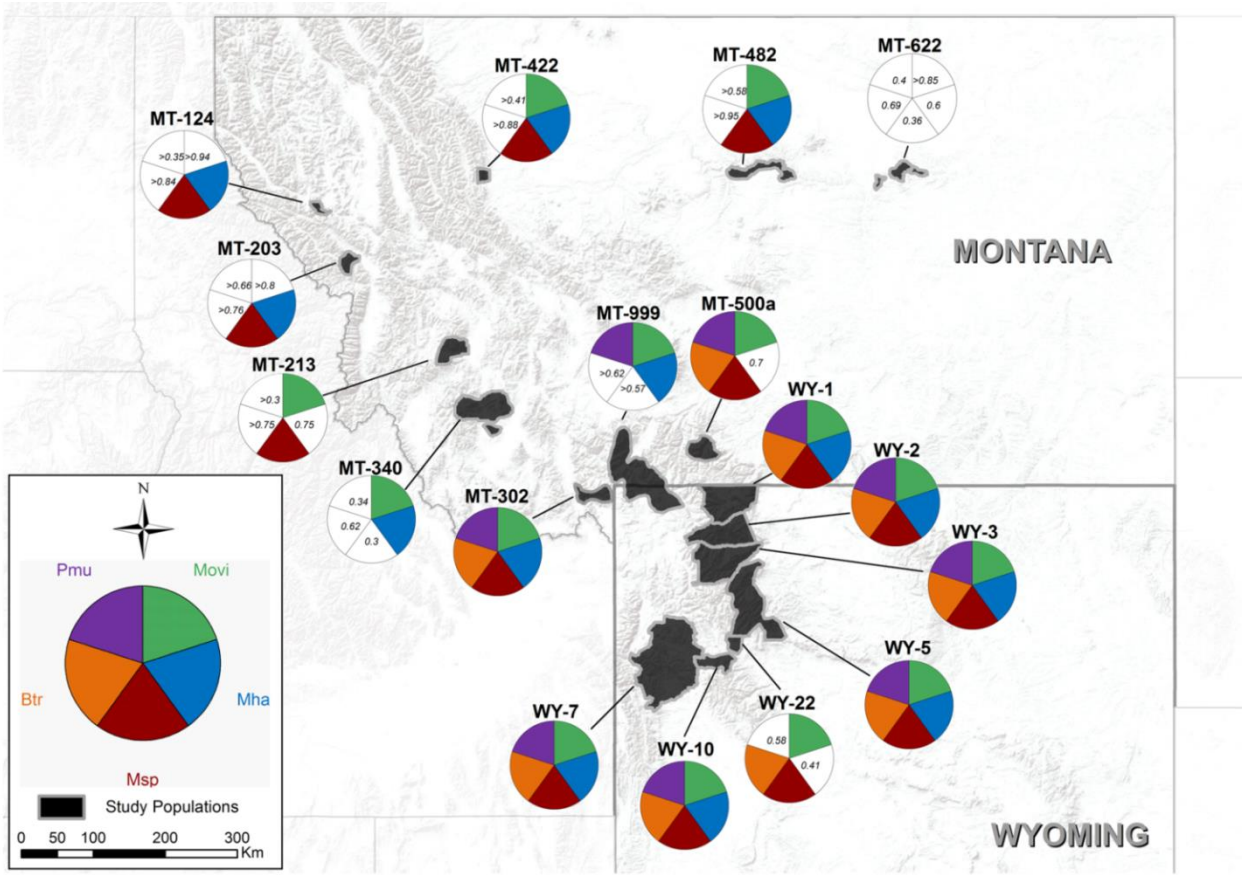


Figure 2.1. Map of bighorn sheep study populations and detected respiratory pathogen communities. All sections of the pie-charts are fixed to equal size and represent whether the respective pathogens were detected in the study population. The key for pathogen abbreviations are as follows: Movi= *Mycoplasma ovipneumoniae,* Mha = leukotoxigenic *Mannheimia haemolytica/glucosida,* Msp = leukotoxigenic *Mannheimia* spp., Btr = leukotoxigenic *Bibersteinia trehalosi,* Pmu = *Pasteurella multocida.* Where pathogens were not detected, the numbers in the unfilled section indicate the power of the employed sampling methodologies to detect the pathogen at 10% prevalence in the population.

Table 2.2 All-age respiratory disease epizootic history, population status relative to management objective, recruitment rates, and population trend of study populations with respect to detection (+) or non-detection (-) of five bighorn sheep respiratory pathogens. Populations where the power to detect a specific pathogen was less than 0.80 are not considered in that pathogen's summary.

	Mycoplasma ovipneumoniae		*Mannheimia haemolytica*[4]		*Mannheimia species*[4]		*Bibersteinia trehalosi*		*Pasteurella multocida*[4]	
	+	-	+	-	+	-	+	-	+	-
# Populations	14	3	13	--	14	--	9	2	9	--
Previous all-age respiratory disease epizootics	64% (9/14)	0% (0/3)	54% (7/13)	--	50% (7/14)	--	56% (5/9)	33% (1/3)	66% (6/9)	--
Meets mgmt. objective[1]	50% (7/14)	100% (3/3)	62% (8/13)	--	50% (7/14)	--	56% (5/9)	66% (2/3)	66% (6/9)	--
Adequate recruitment[2]	79% (10/13)	100% (3/3)	92% (11/12)	--	79% (11/14)	--	89% (8/9)	66% (2/3)	100% (9/9)	--
Trend stable or growing[3]	62% (8/13)	100% (3/3)	75% (9/12)	--	64% (9/14)	--	89% (6/9)	66% (2/3)	78% (7/9)	--

[1.] Meeting population objective defined as ≥ 80% of listed population objective for Wyoming populations and ≥ 90% of listed population objective for Montana populations pursuant to state and population-specific management goals.
[2.] Adequate recruitment defined as average lamb:ewe ratios > 0.20 [38].
[3.] Based on professional opinion of ten-year population trend.
[4] Presence of *Mannheimia haemolytica, Mannheimia species,* or *Pasteurella multocida* was never reliably assessed in populations where they were not detected.

after which the average lamb:ewe ratio was different. Break-years only occurred within the recruitment analysis time-series (after 2011) for three populations (Clark's Fork-2014, Hilgard-2012, Stillwater-2013, and Whiskey Basin-2012; Appendix F, Table F2).

After censoring data and splitting populations according to documented all-age respiratory epizootics, 91 population-years of recruitment data (lamb:ewe ratios) from 18 populations were available to assess differences in mean lamb:ewe ratios with respect to the detection of *M. ovipneumoniae* or *B. trehalosi.* The number of records available for the *B. trehalosi* analysis was further reduced to 66 according to the number of populations where pathogens were not detected and adequate detection power (≥0.80) was not achieved. Due to poor confidence in negative test results for all populations where *M. haemolytica*, *Mannheimia* spp., *or P. multocida* were not detected (Figure 2.1), regression analyses were not conducted for these pathogens and lamb:ewe ratios were summarized for the populations where they were detected.

Mean lamb:ewe ratios of individual populations where any specific pathogen was detected ranged from less than 0.20 to greater than 0.40 (Table 2.3, Figure 2.2). For each pathogens species, there were at least five populations that hosted it and had mean lamb:ewe ratios greater than 0.30. Mean lamb:ewe ratios of populations where *M. ovipneumoniae* was not detected were 0.34 (Perma-Paradise, 0.44 (Petty Creek), and 0.47 (Middle Missouri Breaks). Mean lamb:ewe ratios for populations where *B. trehalosi* was not detected (and adequate detection power was achieved) were 0.09 (Castle Reef), 0.29 (Fergus), and 0.34 (Paradise). There was evidence for an association between detection of *M. ovipneumoniae* and lamb:ewe ratios ($\chi^2_1 = 3.46$, p-value = 0.05). In populations where

M. ovipneumoniae was detected, the estimated mean lamb:ewe ratio was 0.25 (95% CI: 0.20-0.31) and in populations where it was not detected the estimated mean lamb:ewe ratio was 0.42 (95% CI: 0.26-0.67; Table 2.3, Figure 2.2). There was no evidence for an association between detection of *B. trehalosi* ($\chi^2_1 = 1.83$, p-value = 0.18). In populations where *B. trehalosi* was detected the estimated mean lamb:ewe ratio was 0.29 (95% CI: 0.22-0.37) and in populations it was not detected the mean lamb:ewe ratio was 0.20 (95% CI: 0.12-0.32). Associations between presence of any leukotoxigenic *Pasteurellaceae* and lamb:ewe ratios were not explored because leukotoxigenic *Pasteurellaceae* were detected in all but one study population. Interactive effects of *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* could not be explored because *M. ovipneumoniae* was never detected in the absence of leukotoxigenic *Pasteurellaceae*, however recruitment data for populations where both were *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* were and were not detected are shown in Figure 2.2.

Population Growth and Pathogen Detection

Following data censoring on account of insufficient trend count data, 119 population surveys from 13 different populations were used in the population growth analysis; however pathogen-specific analyses further excluded data from populations where that pathogen's absence was not reliably determined. Variation in sightability between 50% and 85% had a small effect on estimates of average (geometric mean) lambda ($\bar{\lambda}$) as the mean difference in estimated $\bar{\lambda}$ between the two sightabilities across all populations was 0.015 (Appendix F, Figure F2). Accordingly, results presented henceforth assume 70% sightability in population surveys, as has been used in recent

Table 2.3. Summary of lamb:ewe ratios in bighorn sheep study populations with respect to detection of five respiratory pathogens. Populations where the power to detect a specific pathogen was less than 0.80 are not considered in that pathogen's summary.

	Mycoplasma ovipneumoniae		*Mannheimia haemolytica*[1]		*Mannheimia species*[1]		*Bibersteinia trehalosi*		*Pasteurella multocida*[1]	
	+	-	+	-	+	-	+	-	+	-
# Populations	14	3	13	0	14	0	9	3	9	0
Mean lamb:ewe ratio (95% CI)	0.26 (0.21-0.32)	0.42 (0.25-0.66)	0.27 (0.22-0.37)	--	0.26 (0.20-0.33)	--	0.29 (0.22-0.39)	0.20 (0.12-0.37)	0.32 (0.24-0.42)	--
Minimum mean population-specific lamb:ewe ratio	0.09 (MT-422)	0.35 (MT-124)	0.09 (MT-422)	--	0.09 (MT-422)	--	0.17 (WY-22)	0.09 (MT-422)	0.17 (WY-22)	--
Maximum mean population-specific lamb:ewe ratio	0.48 (MT-500a)	0.48 (MT-622)	0.44 (MT-302)	--	0.48 (MT-500a)	--	0.48 (MT-500a)	0.34 (MT-124)	0.48 (MT-500a)	--

[1.] Presence of *Mannheimia haemolytica, Mannheimia species,* or *Pasteurella multocida* was never reliably assessed in populations where they were not detected.

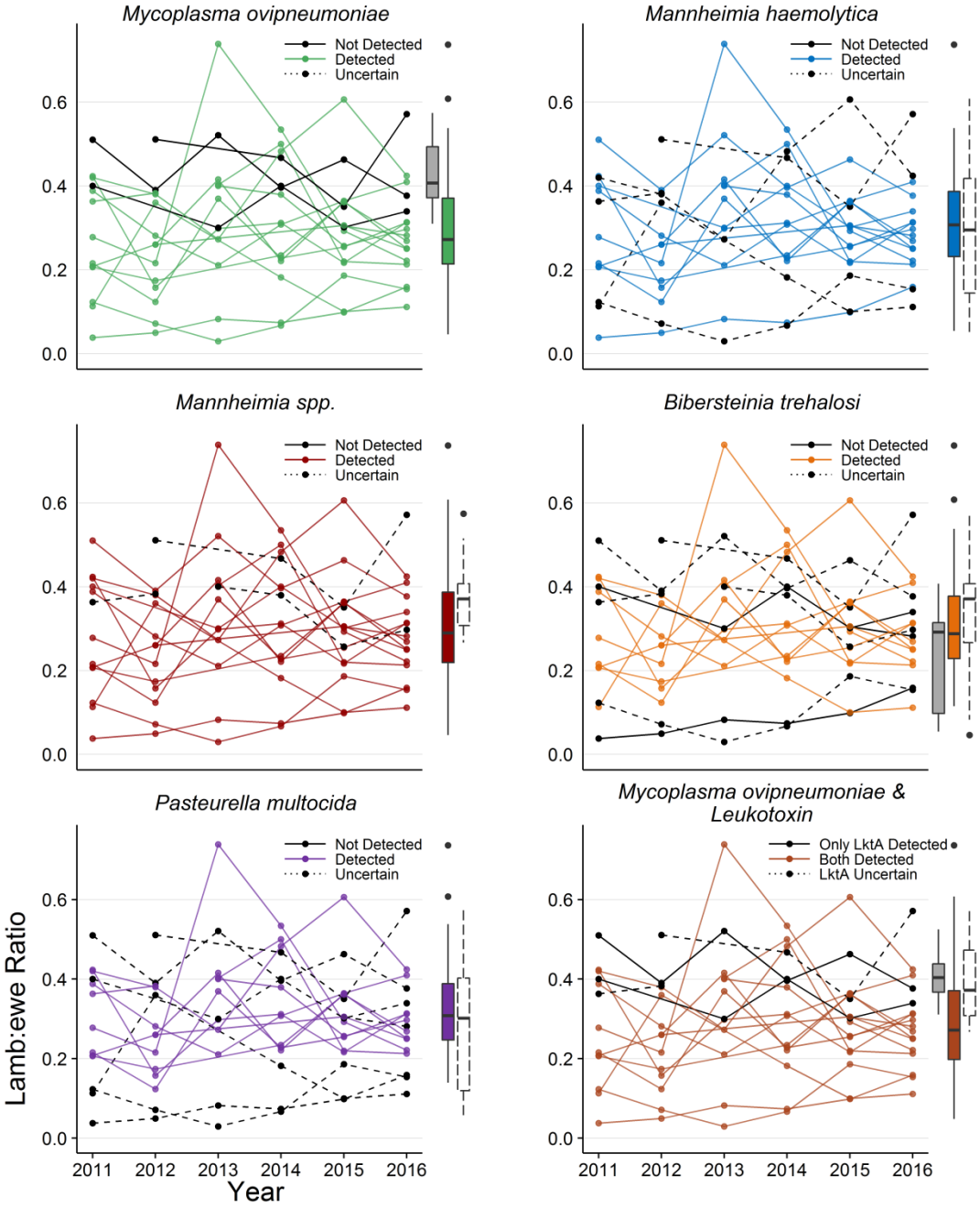


Figure 2.2. Lamb:ewe ratios of 17 bighorn sheep populations in Montana and Wyoming that were investigated in this study with respect to detection status of five respiratory pathogens. The y-axes show observed lamb:ewe ratios (lambs:100 ewes) and the main x-axes show years the data were collected. Grey boxplots show lamb:ewe ratios of populations where the respective pathogen was not detected and there was strong (>80%) detection power, colored boxplots show where the respective pathogen was detected and unfilled boxplots show where the respective pathogen was not detected but there was poor (<80%) power to detect it.

investigations of mountain ungulate population growth rates [60]. Eight of the 13 (62%) populations in the population growth analysis were estimated to have positive population growth ($\bar{\hat{\lambda}} > 1$) and five (38%) were estimated to have negative population growth rates ($\bar{\hat{\lambda}} < 1$). However, the 95% confidence interval for $\bar{\lambda}$ of every population overlapped 1 (Appendix F, Figure F2). The estimated confidence level that $\bar{\lambda} > 1$ (i.e., probability $\bar{\hat{\lambda}} | \bar{\lambda} > 1$) for individual populations ranged from 0.13 (Castle Reef) to 0.95 (Fergus).

Average estimated geometric population growth ($\bar{\hat{\lambda}}$) of individual populations where any specific pathogen was detected ranged from less than 0.95 to greater than 1.10 (Table 2.4). Average lambda ($\bar{\hat{\lambda}}$) estimates for populations where *M. ovipneumoniae* was not detected were 1.01 (95% CI: 0.88-1.16) for Perma-Paradise, 1.06 (95% CI: 0.90-1.24) for Petty Creek, and 1.08 (95% CI: 0.90-1.30) for Middle Missouri Breaks. Average lambda estimates for populations where *B. trehalosi* was not detected (and detection power was at least 0.80) were 0.87 (95% CI: 0.66-1.15) for Castle Reef, 1.01 (95% CI: 0.90-1.24) for Perma-Paradise, and 1.10 (95% CI: 0.97-1.24) for Fergus (Table 2.4, Figure 2.3). Wilcoxon rank-sum tests found no evidence that the confidence levels for positive population growth (i.e., probability $\bar{\hat{\lambda}} | \bar{\lambda} > 1$) was different between populations where either *M. ovipneumoniae* or *B. trehalosi* was and was not detected (*M. ovipneumoniae*: $W_{3,10} = 21$, p-value = 0.37; *B. trehalosi*: $W_{2,9} = 9$, p-value = 0.99).

Discussion

The most important finding of this study may be how pervasive the respiratory pathogens were among the 17 bighorn sheep populations investigated. Intensive sampling

Table 2.4. Summary of geometric population growth in bighorn sheep study populations with respect to detection of five respiratory pathogens. Populations where the power to detect a specific pathogen was less than 0.80 are not considered in that pathogen's summary.

	Mycoplasma ovipneumoniae		*Mannheimia haemolytica*[1]		*Mannheimia species*[1]		*Bibersteinia trehalosi*		*Pasteurella multocida*[1]	
	+	-	+	-	+	-	+	-	+	-
# Populations	14	3	13	0	14	0	9	3	9	0
Median confidence $\bar{\lambda} > 1$	0.51	0.77	0.56	--	0.56	--	0.51	0.56	0.66	--
Median $\hat{\bar{\lambda}}$	1.00	1.06	1.01	--	1.01	--	1.00	1.01	1.04	--
Minimum population-specific $\hat{\bar{\lambda}}$	0.80 (MT-213)	1.01 (MT-124)	0.87 (MT-422)	--	0.80 (MT-213)	--	0.86 (WY-22)	0.87 (MT-422)	0.92 (WY-5)	--
Maximum population-specific $\hat{\bar{\lambda}}$	1.17 (MT-302)	1.08 (MT-622)	1.17 (MT-302)	--	1.17 (MT-302)	--	1.17 (MT-302)	1.10 (MT-482)	1.17 (MT-302)	--

[1.] Presence of *Mannheimia haemolytica*, *Mannheimia species*, or *Pasteurella multocida* was never reliably assessed in populations where they were not detected.

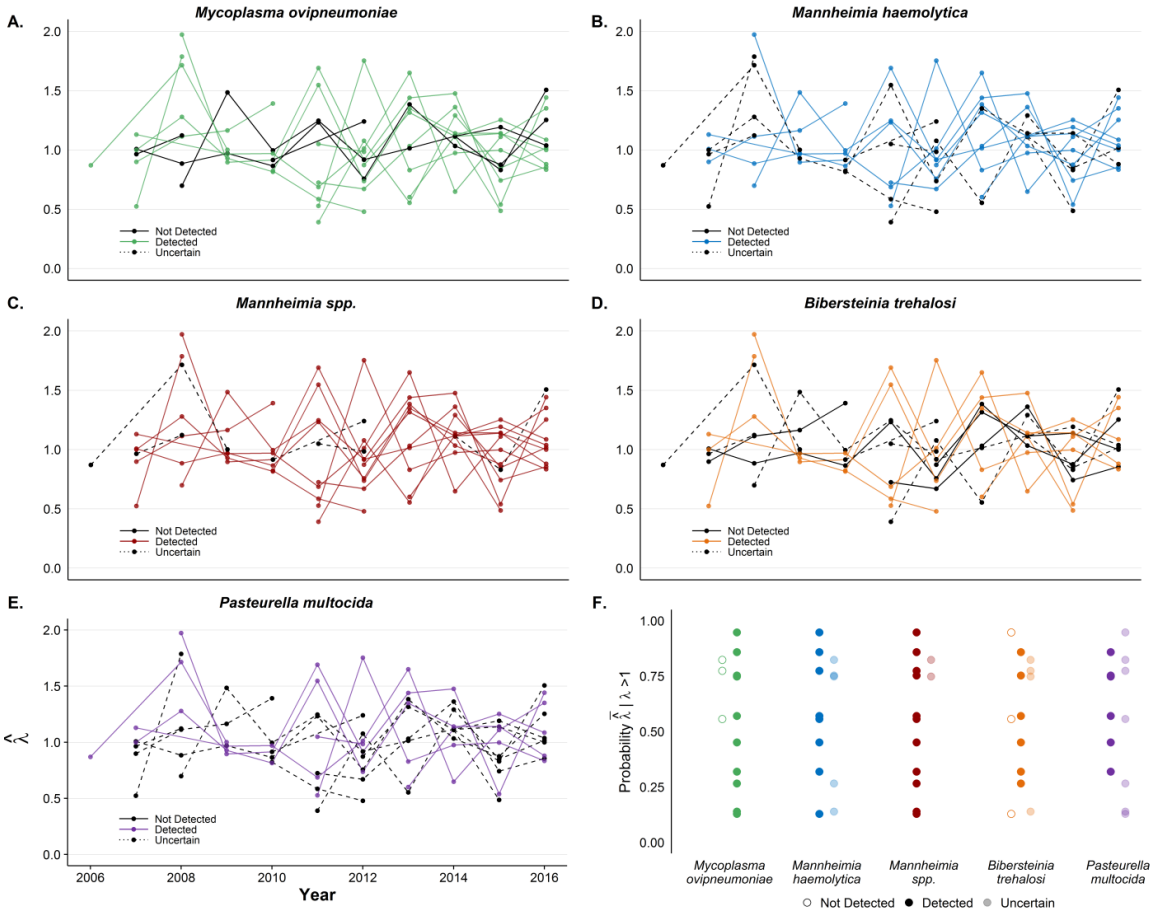


Figure 2.3. Estimates of annual population growth rates and confidence levels for positive population growth in 17 bighorn sheep populations in Montana and Wyoming. Panels A-E show annual lambda λ estimates, where estimable, for 13 bighorn sheep populations in Montana and Wyoming, with respect to detection status of five respiratory pathogens. The y-axes of panels A-E show λ estimates and the x-axes show years. Panel F shows estimated confidence level for positive population growth (i.e., probability $\bar{\hat{\lambda}}|\ \bar{\lambda} > 1$) of the 13 bighorn sheep populations on the y-axis with respect to whether or not five respiratory pathogens were detected.

found the two most cited agents responsible for respiratory disease, *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae*, were both present in at least 76% of the study populations within which nearly 6,000 bighorn sheep live. These pathogens are hosted in bighorn sheep populations that inhabit matrices of public and private land as well as those in the most remote areas of the continental United States that offer the most stringent protections against human and livestock. These findings demonstrate that the combination of bighorn sheep ecology and anthropogenic use of the landscape results in a propensity for bighorn sheep across a variety of landscapes, including national parks and wilderness areas, to be exposed to these respiratory pathogens. It is not known how long the study populations have hosted these respiratory pathogens. Accordingly, it is not known the extent to which the current pervasiveness of these pathogens in the populations is the result of continued "spillover" events from domestic livestock despite concerted efforts to prevent contact between the species' or the result of past eras when domestic sheep were ubiquitous across bighorn sheep range. Regardless, the fact that 76% of the study populations, including the most abundant populations, host both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae*, highlights the substantial, landscape-level challenges that wildlife agencies have faced and continue to face in preventing the spread of pathogens to bighorn sheep populations. Recent evidence suggests that acquired immunity of bighorn sheep to *M. ovipneumoniae* is strain-specific [61], indicating that exposure to new pathogen strains introduced from domestic livestock, neighboring bighorn sheep populations, translocated bighorn sheep, and even sympatric mountain

goats (*Oreamnos americanus*) [62] pose a risk of respiratory disease for any bighorn sheep population even if it already hosts pathogen species linked to respiratory disease.

Although both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* were detected in most (n=14) study populations, these study populations often showed no demographic signs of respiratory disease. Half of the populations where these pathogens were detected met population objectives and had positive estimated growth rates (though never statistically significant), ten (77%) had average lamb:ewe ratios greater than 0.20 (threshold for "healthy" recruitment defined by the Western Association of Fish and Wildlife Agencies), and six had average lamb:ewe ratios greater than 0.30. Generally, this group of populations included those with the lowest and highest population sizes, population growth rates, and average recruitment rates. The number of populations found to host *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* and the variation in demographic performance among these populations resulted in the paradoxical finding that, although average demographic performance in this group of populations was lower than where *M. ovipneumoniae* was not detect, most populations that were estimated to have positive growth rates and average recruitment rates greater than 0.30 were ones that carried both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae.* At face value, this pattern suggests that bighorn sheep populations can be successfully managed while hosting all respiratory pathogens that have been tied to respiratory disease. However, the significance of this pattern hinges on whether the collection of study populations here is representative of bighorn sheep populations as a whole and what explains the variation in demographic performance of populations hosting apparently similar pathogen

communities. Although the study populations were not randomly selected, they were chosen to capture a wide range of variability in herd attributes in order to maximize the generalizability of the findings.

The strong demographic performance of some populations hosting *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* could be explained by the presence of less virulent pathogen strains which cannot be distinguished by the available diagnostic tests [1,18]. Differences in virulence could be inherent in the strains or attenuated after years of persistence in bighorn sheep populations. It is known that the study populations carry different strains of *M. ovipneumoniae* (Montana State University unpublished data), yet not whether the strains vary in virulence. Available diagnostic tests for *Pasteurellaceae* are unable to reliably distinguish among species, particularly in the *Mannheimia* genus (Wyoming Game and Fish Department unpublished data, [18]), leaving little ability to discern whether *Pasteurellaceae* hosted by different study populations vary in virulence. Variation in demographic performance could also be explained by differences in prevalence of *M. ovipneumoniae* or leukotoxigenic *Pasteurellaceae,* which was not assessed here. Prevalence was not assessed in this investigation because it was previously found (chapter one) that this parameter is likely estimated with poor precision in the face of imperfect detection probability, particularly for *Pasteurellacea.* Variation in *M. ovipneumoniae* prevalence at the time of testing does not appear to explain variation in demographic performance in a direct way, as *M. ovipneumoniae* was detected in only six (6) of 30 animals sampled from the population with the lowest average recruitment rate (Castle Reef) but was detected in 24 of 29

animals and 28 of 50 animals in the Hilgard population during two years when estimated recruitment rates were 0.53 and 0.29 respectively. Comparing prevalence of *Pasteurellaceae* across study populations is untenable given low detection probability for most diagnostic protocols.

If variation in demographic rates within the group of populations hosting both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* is not explained by unobserved differences in pathogen communities, the variation of demographic rates, and presumably disease expression may be dictated by interactions between the pathogen, the host, and the environment. Favorable demographics of some populations may be temporary states enjoyed for a limited time until conditions sufficient for expression of disease are met. The environment experienced by some populations may be less likely to produce conditions sufficient for expression of disease, leading to differential population performance given the same respiratory pathogen community and inherent per-animal disease resilience. Consequently, subsequent respiratory disease epizootics in these populations may be due to introduction of novel pathogen strains or increased expression or transmission of resident pathogens [4,5]. Given variable population-management histories and over a century of exposure to domestic sheep experienced by some populations, selection may have produced increased disease resilience in certain study populations. High adult and juvenile mortality rates associated with respiratory disease suggest potential for strong selective pressure for physiological or behavioral adaptations against respiratory disease so long as surviving individuals are exposed to the causative agent, traits associated with survival are heritable, and sufficient genetic variability exists.

While obvious signs of selection for disease resistance are absent, selection results in local adaptation, for which there is evidence in bighorn sheep [63], and outbreeding from extensive translocations may interfere with selection and local adaptation [64].

Many of the larger study populations that host both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* are metapopulations comprised of numerous sub-populations that may behave independently and have asynchronous demographic rates despite being in close proximity. The generally satisfactory measurements of demographic performance across the metapopulation within which pathogens were detected may be an average of disparate sub-populations that individually did not host the same respiratory pathogens or did not similarly express respiratory disease at the time of study. Recent research demonstrated this asynchrony in lamb survival across sub-populations of a bighorn sheep population with chronic lamb pneumonia [65]. The scale at which the pathogen and demographic data in this study were summarized could then mask significant effects of respiratory disease in some local sub-populations with the demographic vigor of other sub-populations that either were not exposed to the same pathogens or were asynchronously expressing disease. Thus, the metapopulation may only give the illusion that these populations are resilient to respiratory disease. However, coarse-scale stability despite asynchrony and instability at a fine scale is the exact mechanism for which management for metapopulations is a well-established practice in the field of conservation biology [66]. Although some evidence exists that individual host-organisms within metapopulations can be more resistant to pathogens despite high potential for pathogen spread [67], the benefits of metapopulations as described by

classic metapopulation theory are not based upon improved performance of any particular individual or sub-population, but extinction and colonization dynamics [68]. Thus, asynchronous population dynamics within a highly-connected bighorn sheep metapopulation where respiratory pathogens reside may illustrate one of the benefits that metapopulations provide for conservation biology.

As a consequence of the pervasiveness of *M. ovipneumoniae* in the study populations, most populations that exhibited adequate demographic performance carried *M. ovipneumoniae*. Despite the low number of populations where *M. ovipneumoniae* was not detected, there was evidence consistent with the hypothesis that this pathogen is a primary etiological agent for respiratory disease in bighorn sheep. For example, none of the three populations where *M. ovipneumoniae* was not detected have a documented history of respiratory disease, compared to nine of 14 populations where it was detected. There was also statistical evidence that average recruitment rates in these populations were higher than in populations where *M. ovipneumoniae* was detected. All three populations where *M. ovipneumoniae* was not detected had average recruitment rates greater than 0.30 and population growth rates greater than one. However, population growth and recruitment rates of the populations where *M. ovipneumoniae* was not detected were not particularly unusual compared to those of numerous populations where it was detected. Collectively, these findings support the hypothesis that *M. ovipneumoniae* is a necessary agent for respiratory disease in bighorn sheep, but do not suggest that its presence in a population is sufficient for population-limiting respiratory

disease. Investigation of additional populations without evidence for exposure to *M. ovipneumoniae* is needed for stronger inference.

After uncertainty in diagnostic test results was quantified, it was found that presence of most *Pasteurellaceae* species could not be associated with demographic rates because there was little ability to determine with confidence in which populations they were not present. For leukotoxigenic *B. trehalosi* (for which there was adequate detection power for several populations) there was no evidence of an association with demographic performance. Additionally poor specificity of diagnostic tests may have misidentified the species of isolates [18], further limiting the ability to link demographic performance to presence of *Pasteurellaceae*. The detection of leukotoxigenic *Pasteurellaceae* in 94% of the study populations combined with low power to detect individual *Pasteurellaceae* species in nearly all populations where they were not detected suggests that leukotoxigenic *Pasteurellaceae* is ubiquitous. If this is true, variation in expression of respiratory disease cannot be explained by leukotoxigenic *Pasteurellaceae* and the associations of *M. ovipneumoniae* with demographic performance in this study are conditional upon presence of leukotoxigenic *Pasteurellaceae.* While associative evidence for *Pasteurellaceae* playing a role in bighorn sheep respiratory disease is weak, experimental evidence strongly suggests leukotoxigenic strains cause severe respiratory disease in bighorn sheep. Thus, leukotoxigenic *Pasteurellaceae* may still pose a risk to bighorn sheep populations, particularly if introduced to populations hosting *M. ovipneumoniae*.

Management Implications

The findings of this study warrant caution for augmenting bighorn sheep populations with animals from distinct populations as there is high probability that a pathogen species linked to respiratory disease will be introduced to the recipient populations. Although certain pathogen species may ultimately be determined to be benign or safe to mix, caution is prudent when uncertainty exists. The common occurrence of *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* in populations exhibiting strong demographic performance also suggests potential that historic translocations introduced novel strains of these pathogens into new host populations.

The same general findings that warrant caution against population augmentation also suggest plausibility in the hypothesis that some proportion of respiratory disease epizootics are caused by resident pathogens already hosted by the affected population. Lack of quantification in the reliability of historic test results and a focus on sampling diseased populations leaves open the question of whether pathogens which were first detected during respiratory disease epizootics were present before the epizootic began. It is imperative to maintain and improve upon policies of separation between bighorn sheep and domestic sheep and goats. However, domestic livestock currently on the landscape may not be the source of all bighorn sheep respiratory disease epizootics. Thus, it is of conservation value to rigorously evaluate evidence for the novel and resident pathogen hypotheses to ensure that efforts to reduce incidence of respiratory disease are directed as effectively as possible [5]. These hypotheses can be addressed by rigorously sampling populations during periods of demographic vigor and subsequently re-sampling those

populations which experience respiratory disease epizootics to assess whether new pathogen strains are detected. Although the case-by case results of this approach are largely circumstantial, the collective results across many populations should lend credible evidence for or against both hypotheses and will either reinforce the current disease management paradigm or promote adoption of novel management approaches. Evidence that multiple study populations host both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* and have strong demographic performance suggests that additional management approaches may exist to reduce the effects of respiratory disease in bighorn sheep by developing management strategies that promote disease resilience. Disease resilience and susceptibility could be explained by habitat quality or environmental characteristics, inherent traits of individual animals, or spatial structuring of populations. Specific hypotheses within these categories are abundant and, as a result, challenging to assess. Because the traits that may influence population resilience vary at the population-level, a large sample of populations, from which reliable data on pathogen communities and herd characteristics can be collected, would be required to elucidate traits associated with population resilience.

Recommendations and Conclusions:

This study rigorously assessed respiratory pathogen communities and demographic rates in 17 bighorn sheep populations across two US states, is the first study to report uncertainty in its pathogen testing results. This study demonstrated that pathogens associated with respiratory disease are pervasive among free-ranging bighorn sheep and numerous populations hosting these pathogens exhibit demographic vigor.

These simple patterns generate some concrete recommendations for translocation policy and interpretation of diagnostic test results but also raise several questions that could lead to additional tools for management of bighorn sheep respiratory disease. The findings demonstrate plausibility for related hypotheses that a) identifiable characteristics of certain bighorn sheep populations related to habitat quality, inherent traits of individuals, or spatial structuring of animals within the populations can provide increased resilience against respiratory disease and b) respiratory disease epizootics can be caused by either introduction of novel pathogens or increased virulence or transmission of resident pathogens that were hosted by populations during previous periods of demographic vigor. Although this study lacks the geographic and temporal scale to address these hypotheses, it provides baseline data and recommendations for future studies that could address these hypotheses and provide additional insights for managing respiratory disease. These recommendations include:

1.) Continue to improve characterization of respiratory pathogen communities by working to adopt diagnostic protocols that provide strong ability to detect pathogens in sampled bighorn sheep populations and identify different strains within pathogen species. Statistical tools should be used to quantify uncertainty in negative test results.

2) Develop a monitoring program for a large and diverse set of bighorn sheep populations where a sufficient number of animals (n=30) can be sampled from each population to assess respiratory pathogen communities. Collect baseline respiratory pathogen data from

populations both with and without indication of respiratory disease and resample populations following disease epizootics using comparable methods.

3) Characterize attributes of study populations that could influence expression of respiratory disease, such as genetics, physiological status, body condition, and spatial structure of sub-populations.

4.) Coordinate and standardize these efforts across states and provinces to collect adequate and comparable data from as many contrasting bighorn sheep populations as possible.

These recommendations aim to provide sufficient information to evaluate how often disease epizootics are associated with novel or resident pathogen strains, whether variation in virulence of pathogen strains can explain demographic vigor in some bighorn sheep populations hosting *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae*, and highlight potential traits associated with population-level resilience against respiratory disease. The collective evidence could then be used to a) validate and reinforce the current emphasis of management policies on minimizing pathogen transmission from domestic livestock and/or b) spur additional investigation into characteristics of populations and their environment that could be manipulated to minimize the demographic effects of respiratory pathogens. Promoting resilience of populations against pervasive respiratory pathogens offers an additional and complementary approach for wildlife agencies to manage bighorn sheep populations in the modern landscape.

REFERENCES CITED

1. Besser TE, Frances Cassirer E, Highland MA, Wolff P, Justice-Allen A, Mansfield K, et al. Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. Prev Vet Med. 2013;108: 85–93. doi:10.1016/j.prevetmed.2012.11.018

2. Cassirer EF, Sinclair ARE. Dynamics of pneumonia in a bighorn sheep metapopulation. J Wildl Manag. 2007;71: 1080–1088. doi:10.2193/2006-002

3. Butler C, Garrott RA, Rotella JJ. Correlates of recruitment in Montana bighorn sheep populations [Internet]. Helena, Montana; 2013 Jul. Available: http://www.wafwa.org

4. Miller M, Hobbs N, Williams E. Spontaneous pasteurellosis in captive Rocky-Mountain bighorn sheep (Ovis-canadensis canadensis) - Clinical, laboratory, and epizootiological observations. J Wildl Dis. 1991;27: 534–542.

5. Miller DS, Hoberg E, Weiser G, Aune K, Atkinson M, Kimberling C. A review of hypothesized determinants associated with bighorn sheep (Ovis canadensis) die-offs. Vet Med Int. 2012;2012: 1–19. doi:10.1155/2012/796527

6. Besser TE, Highland M, Baker K, Cassirer EF, Anderson NJ, Ramsey JM, et al. Causes of pneumonia epizootics among bighorn sheep, western United States, 2008–2010. Emerg Infect Dis. 2012;18: 406–414. doi:10.3201/eid1803.111554

7. Shanthalingam S, Goldy A, Bavananthasivam J, Subramaniam R, Batra SA, Kugadas A, et al. PCR assay detects Mannheimia haemolytica in culture-negative pneumonic lung tissues of bighorn sheep (Ovis canadensis) from outbreaks in the western USA, 2009-2010. J Wildl Dis. 2014;50: 1–10. doi:10.7589/2012-09-225

8. Besser TE, Cassirer EF, Potter KA, Lahmers K, Oaks JL, Shanthalingam S, et al. Epizootic pneumonia of bighorn sheep following experimental exposure to Mycoplasma ovipneumoniae. PLoS ONE. 2014;9: e110039. doi:10.1371/journal.pone.0110039

9. Dassanayake RP, Shanthalingam S, Subramaniam R, Herndon CN, Bavananthasivam J, Haldorson GJ, et al. Role of Bibersteinia trehalosi, respiratory syncytial virus, and parainfluenza-3 virus in bighorn sheep pneumonia. Vet Microbiol. 2013;162: 166–172. doi:10.1016/j.vetmic.2012.08.029

10. Wood ME, Fox KA, Jennings-Gaines J, Killion HJ, Amundson S, Miller MW, et al. How Respiratory Pathogens Contribute to Lamb Mortality in a Poorly Performing Bighorn Sheep (Ovis canadensis) Herd. J Wildl Dis. 2016; doi:10.7589/2016-05-097

11. Drew ML, Edwards WH, Fox KA, Griffin CM, Gonzales BJ, Jennings-Gaines JE, et al. WAFWA wildlife health committee 2014: Bighorn sheep herd health monitoring recommendations. Western Association of Fish and Wildlife Agencies; 2015.

12. Carlsen T, Erickson G. Montana bighorn sheep conservation strategy [Internet]. Helena, Montana; 2010 Jan. Available: http://fwp.mt.gov/fishAndWildlife/management/bighorn/plan.html#plan

13. Dassanayake RP, Call DR, Sawant AA, Casavant NC, Weiser GC, Knowles DP, et al. Bibersteinia trehalosi inhibits the growth of Mannheimia haemolytica by a proximity-dependent mechanism. Appl Environ Microbiol. 2010;76: 1008–1013. doi:10.1128/AEM.02086-09

14. Walsh DP, Wolfe LL, Vieira MEP, Miller MW. Detection probability and Pasteurellaceae surveillance in bighorn sheep. J Wildl Dis. 2012;48: 593–602.

15. Bavananthasivam J, Dassanayake RP, Kugadas A, Shanthalingam S, Call DR, Knowles DP, et al. Proximity-dependent inhibition of growth of Mannheimia haemolytica by Pasteurella multocida. Appl Environ Microbiol. 2012;78: 6683–6688. doi:10.1128/AEM.01119-12

16. Safaee S, Weiser GC, Cassirer EF, Ramey RR, Kelley ST. Microbial diversity in bighorn sheep revealed by culture-independent methods. J Wildl Dis. 2006;42: 545–555. doi:10.7589/0090-3558-42.3.545

17. Fisher MA, Weiser GC, Hunter DL, Ward ACS. Use of a polymerase chain reaction method to detect the leukotoxin gene IktA in biogroup and biovariant isolates of Pasteurella haemolytica and P trehalosi. Am J Vet Res. 1999;60: 1402–1406.

18. Miller MW, Hause BM, Killion HJ, Fox KA, Edwards WH, Wolfe LL. Phylogenetic and epidemiologic relationships among Pasteurellaceae from Colorado bighorn sheep herds. J Wildl Dis. 2013;49: 653–660. doi:10.7589/2012-11-274

19. MacKenzie DI, Nichols JD, Lachman GB, Droege S, Andrew Royle J, Langtimm CA. Estimating site occupancy rates when detection probabilities are less than one. Ecology. 2002;83: 2248–2255. Available: http://www.esajournals.org/doi/abs/10.1890/0012-9658(2002)083%5B2248:ESORWD%5D2.0.CO%3B2

20. Fiske I, Chandler R. unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. J Stat Softw. 2011;43: 1–23. Available: http://ag.arizona.edu/classes/wfsc578/Fiske%20and%20Chandler%202011.pdf

21. R Core Team. R: a language and environment for statistical computing. R Foundation for Statisical Development; 2016.

22. Christensen RHB, Christensen MRHB. Package “ordinal.” 2015; Available: http://mirrors-usa.go-parts.com/cran/web/packages/ordinal/ordinal.pdf

23. MacKenzie DI, Bailey LL. Assessing the fit of site-occupancy models. J Agric Biol Environ Stat. 2004;9: 300–318. doi:10.1198/108571104X3361

24. Haberman SJ. A Warning on the Use of Chi-Squared Statistics with Frequency Tables with Small Expected Cell Counts. J Am Stat Assoc. 1988;83: 555–560. doi:10.1080/01621459.1988.10478632

25. Ellis MM, Ivan JS, Schwartz MK. Spatially Explicit Power Analyses for Occupancy-Based Monitoring of Wolverine in the U.S. Rocky Mountains: Power in Occupancy-Based Trend Detection. Conserv Biol. 2014;28: 52–62. doi:10.1111/cobi.12139

26. Wild MA, Miller MW. Effects of modified cairy and blair medium on recovery on nonhemolytic pasteurella haemolytica from Rocky Mountain bighorn sheep (Ovis canadensis canadensis) pharyngeal swabs. J Wildl Dis. 1994;30: 16–19. doi:10.7589/0090-3558-30.1.16

27. Miller DAW, Talley BL, Lips KR, Campbell Grant EH. Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs: Detection in epidemiological studies. Methods Ecol Evol. 2012;3: 850–859. doi:10.1111/j.2041-210X.2012.00216.x

28. Sergeant E. Epitools epidemiological calculators [Internet]. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease; Available: http://epitools.ausvet.com.au

29. Besser TE, Cassirer EF, Potter KA, VanderSchalie J, Fischer A, Knowles DP, et al. Association of Mycoplasma ovipneumoniae infection with population-limiting respiratory disease in free-ranging Rocky Mountain bighorn Sheep (Ovis canadensis canadensis). J Clin Microbiol. 2008;46: 423–430. doi:10.1128/JCM.01931-07

30. Lachish S, Gopalaswamy AM, Knowles SCL, Sheldon BC. Site-occupancy modelling as a novel framework for assessing test sensitivity and estimating wildlife disease prevalence from imperfect diagnostic tests. Methods Ecol Evol. 2012;3: 339–348. doi:10.1111/j.2041-210X.2011.00156.x

31. Picton HD, Lonner TN. Montana's wildlife legacy: decimation to restoration. Media Works Pub; 2008.

32. Toweill DE, Geist V. Return of Royalty: Wild Sheep of North America. Boone and Crockett Club; 1999.

33. Johnson HE, Hebblewhite M, Stephenson TR, German DW, Pierce BM, Bleich VC. Evaluating apparent competition in limiting the recovery of an endangered ungulate. Oecologia. 2013;171: 295–307. doi:10.1007/s00442-012-2397-6

34. Festa-Bianchet M, Coulson T, Gaillard J-M, Hogg JT, Pelletier F. Stochastic predation events and population persistence in bighorn sheep. Proc R Soc B Biol Sci. 2006;273: 1537–1543. doi:10.1098/rspb.2006.3467

35. Hogg JT, Forbes SH, Steele BM, Luikart G. Genetic rescue of an insular population of large mammals. Proc R Soc B Biol Sci. 2006;273: 1491–1499. doi:10.1098/rspb.2006.3477

36. Johnson HE, Scott Mills L, Wehausen JD, Stephenson TR. Combining ground count, telemetry, and mark-resight data to infer population dynamics in an endangered species: Combining data types to infer dynamics. J Appl Ecol. 2010;47: 1083–1093. doi:10.1111/j.1365-2664.2010.01846.x

37. Kramer AM, Dennis B, Liebhold AM, Drake JM. The evidence for Allee effects. Popul Ecol. 2009;51: 341. doi:10.1007/s10144-009-0152-6

38. Brewer C, Bleich VC, Foster J, Hosch-Hebdon T, McWhirter D, Rominger E, et al. Bighorn sheep conservation challenges: Management strategies for the 21st Century. Cheyenne, Wyoming, USA: Western Association of Fish and Wildlife Agencies; 2014 Jul pp. 1–32.

39. Lawrence PK, Shanthalingam S, Dassanayake RP, Subramaniam R, Herndon CN, Knowles DP, et al. Transmission of Mannheimia haemolytica from domestic sheep (Ovies aries) to bighorn sheep (Ovis canadensis): Unequivocol demonstration with green fluorescent protein-tagged organisms. J Wildl Dis. 2010;46: 706–717.

40. Wehausen JD, Kelley ST, Ramey RR. Domestic sheep, bighorn sheep, and respiratory disease: a review of the experimental evidence. Calif Fish Game. 2011;97: 7–24. Available: http://www.bio.sdsu.edu/faculty/kelley/47.pdf

41. Foreyt WJ. Fatal Pasteurella haemolytica pneumonia in bighorn sheep after direct contact with clinically normal domestic sheep. Am J Vet Res. 1989;50: 341–344.

42. George JL, Martin DJ, Lukacs PM, Miller MW. Epidemic pasteurellosis in a bighorn sheep population coinciding with the appearance of a domestic sheep. J Wildl Dis. 2008;44: 388–403. doi:10.7589/0090-3558-44.2.388

43. Besser TE, Cassirer EF, Yamada C, Potter KA, Herndon C, Foreyt WJ, et al. Survival of bighorn sheep (Ovis canadensis) commingled with domestic sheep (Ovis aries) in the absence of Mycoplasma ovipneumoniae. J Wildl Dis. 2012;48: 168–172. doi:10.7589/0090-3558-48.1.168

44. Dassanayake RP, Shanthalingam S, Herndon CN, Subramaniam R, Lawrence PK, Bavananthasivam J, et al. Mycoplasma ovipneumoniae can predispose bighorn sheep to fatal Mannheimia haemolytica pneumonia. Vet Microbiol. 2010;145: 354–359. doi:10.1016/j.vetmic.2010.04.011

45. Buechner HK. The bighorn sheep in the United States, its past, present, and future. Wildl Monogr. 1960; 3–174.

46. Miller DS, Weiser GC, Ward ACS, Drew ML, Chapman PL. Pasteurellaceae isolated from bighorn sheep (Ovis canadensis) from Idaho, Oregon, and Wyoming. Am J Vet Res. 2012;73: 1024–1028.

47. Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M, Getz WM, Latto J, et al. Should we expect population thresholds for wildlife disease? Trends Ecol Evol. 2005;20: 511–519. doi:10.1016/j.tree.2005.07.004

48. Rachowicz LJ, Hero J-M, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, et al. The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. Conserv Biol. 2005;19: 1441–1448. doi:10.1111/j.1523-1739.2005.00255.x

49. Daszak P, Cunningham AA, Hyatt AD. Emerging Infectious Diseases of Wildlife--Threats to Biodiversity and Human Health. Science. 2000;287: 443–449. doi:10.1126/science.287.5452.443

50. Singer FJ, Williams E, Miller MW, Zeigenfuss LC. Population growth, fecundity, and survivorship in recovering populations of bighorn sheep. Restor Ecol. 2000;8: 75–84. doi:10.1046/j.1526-100x.2000.80067.x

51. Cassirer EF, Plowright RK, Manlove KR, Cross PC, Dobson AP, Potter KA, et al. Spatio-temporal dynamics of pneumonia in bighorn sheep. J Anim Ecol. 2013;82: 518–528. doi:10.1111/1365-2656.12031

52. Manlove K, Cassirer EF, Cross PC, Plowright RK, Hudson PJ. Disease introduction is associated with a phase transition in bighorn sheep demographics. Ecology. 2016;97: 2593–2602. doi:10.1002/ecy.1520

53. Stephen C. Toward a modernized definition of wildlife health. J Wildl Dis. 2014;50: 427–430. doi:10.7589/2013-11-305

54. USDA handbook 296: land resource regions and major land resource areas of the United States, the Caribbean, and the Pacific Basin. United States Department of Agriculture, Natural Resource Conservation Service; 2006.

55. Festa-Bianchet M. Seasonal Dispersion of Overlapping Mountain Sheep Ewe Groups. J Wildl Manag. 1986;50: 325–330. doi:10.2307/3801922

56. Bates DM. lme4: Mixed-effects modeling with R. URL Httplme4 R-Forge R-Proj Orgbook. 2010; Available: http://lme4.0.r-forge.r-project.org/lMMwR/lrgprt.pdf

57. Eberhardt LL. Population projections from simple models. J Appl Ecol. 1987;24: 103–118. doi:10.2307/2403790

58. Skalski JR, Ryding KE, Millspaugh J. Wildlife demography: analysis of sex, age, and count data. Academic Press; 2010.

59. Bodie WL, Garton EO, Taylor ER, McCoy M. A Sightability Model for Bighorn Sheep in Canyon Habitats. J Wildl Manag. 1995;59: 832. doi:10.2307/3801964

60. Flesch EP, Garrott RA, White PJ, Brimeyer D, Courtemanch AB, Cunningham JA, et al. Range expansion and population growth of non-native mountain goats in the Greater Yellowstone Area: Challenges for management. Wildl Soc Bull. 2016;40: 241–250. doi:10.1002/wsb.636

61. Cassirer EF, Manlove KR, Plowright RK, Besser TE. Evidence for strain-specific immunity to pneumonia in bighorn sheep. J Wildl Manag. 2016; n/a-n/a. doi:10.1002/jwmg.21172

62. Wolff PL, Besser TE, Nelson D, Ridphat J, McMullen K, Munoz-Gutierrez J, et al. Mountain goats at the livestock-wildlife interface: A susceptible species. Proceedings Bienniel Symposium of the Northern Wild Sheep and Goat Council. Fort Collins, CO, USA: Northern Wild Sheep and Goat Council; 2014. p. 13. Available: http://media.nwsgc.org/proceedings/NWSGC-2014/013_Wolff-et-al_2014.pdf

63. Wiedmann BP, Sargeant GA. Ecotypic variation in recruitment of reintroduced bighorn sheep: implications for translocation. J Wildl Manag. 2014;78: 394–401. doi:10.1002/jwmg.669

64. Sexton JP, Hangartner SB, Hoffmann AA. Genetic Isolation by Environment or Distance: Which Pattern of Gene Flow Is Most Common? Evolution. 2014;68: 1–15. doi:10.1111/evo.12258

65. Manlove KR, Cassirer EF, Cross PC, Plowright RK, Hudson PJ. Costs and benefits of group living with disease: a case study of pneumonia in bighorn lambs (Ovis canadensis). Proc R Soc B Biol Sci. 2014;281: 20142331–20142331. doi:10.1098/rspb.2014.2331

66. Hanski I. Metapopulation dynamics. Nature. 1998;396: 41–49. doi:10.1038/23876

67. Jousimo J, Tack AJM, Ovaskainen O, Mononen T, Susi H, Tollenaere C, et al. Ecological and evolutionary effects of fragmentation on infectious disease dynamics. Science. 2014;3Thus44: 1289–1293. doi:10.1126/science.1253621

68. Levins R. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull Entomol Soc Am. 1969;15: 237–240. doi:10.1093/besa/15.3.237

69. Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan P. Veterinary Microbiology and Microbial Disease. John Wiley & Sons; 2011.

70. Angen O, Thomsen J, Larsen LE, Larsen J, Kokotovic B, Heegaard PMH, et al. Respiratory disease in calves: Microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. Vet Microbiol. 2009;137: 165–171. doi:10.1016/j.vetmic.2008.12.024

71. McAuliffe L, Ellis RJ, Ayling RD, Nicholas RAJ. Differentiation of Mycoplasma species by 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis fingerprinting. J Clin Microbiol. 2003;41: 4844–4847.

72. Delignette-Muller ML, Dutang C, others. fitdistrplus: An R package for fitting distributions. J Stat Softw. 2015;64: 1–34. Available: http://cran.dataguru.cn/web/packages/fitdistrplus/vignettes/paper2JSS.pdf

73. Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. Prev Vet Med. 1998;34: 1–17. doi:10.1016/S0167-5877(97)00081-0

74. Crawley MJ. Regression. The R Book. John Wiley & Sons, Ltd; 2012. pp. 449–497. Available: http://onlinelibrary.wiley.com/doi/10.1002/9781118448908.ch10/summary

APPENDICES

APPENDIX A

PATHOGEN DIAGNOSTIC PROTOCOL DESCRIPTIONS

Pasteurellaceae:

Wyoming: Cultures from the tonsils were obtained using sterile polyester-tipped applicators (Puritan#25-806 1PD, Guilford, ME, USA) applied to the tonsillar crypts and the outer tonsil surface. Inoculated swabs were immediately used to inoculate one quarter of a Columbia Blood Agar plate (CBA) with 5 % sheep blood (Hardy Diagnostics #A16, Santa Maria, CA, USA). The applicator was then used to resample the tonsil, followed by placement into transport media; Port-A-Cul™ tubes(Becton Dickinson, Franklin Lakes, NJ, USA) or 3ml Amies media without charcoal in a 15 x 103mm culture tube (Triforest Enterprises, Irvine, CA, USA). Samples from the nasal passages were collected using the same type of applicator, but was gently inserted 8-12cm into the nasal cavity while slowly rotating the shaft. The inoculated swab was then placed into transport media as described above. All samples were transported to Wyoming Game and Fish Wildlife Health Laboratory and processed within four hours of collection. Bacterial plates were struck to three quadrants using a 1µm loop and incubated at 37°C in 5% CO_2. Tonsil swabs were removed aseptically from the transport media with forceps and used to inoculate one half of a CBA plate. Tissues were aseptically removed from 18oz Whirl-Pak® bags (Nasco, Fort Atkinson, WI, USA), cut to expose an interior area of tissue, then smeared over half of a CBA plate. The plate was struck to two quadrants for isolation and incubated at 37°C in 5% CO_2. Culture plates were read and documented once at ~18-24 hours, and again at ~36-48 hours. Targeted colonies were recultured for isolation and identified using standard biochemical tests [69]. After 48 hours, all bacterial growth on plate was collected with a polyester-tipped applicator and placed in 15ml

Falcon tubes (Corning, Corning, NY USA) filled with sterile phosphate buffered saline (BBL FTA Hemagglutination, Buffer Becton Dickinson, Franklin Lakes, NJ, USA), and vortexed to suspended bacteria. A 250µl aliquot was removed and placed into a PCR tube (PCR clean 1.5 mL safe-lock tubes Eppendorf, Hauppauge, NY USA) for DNA extraction (E.Z.N.A. Tissue DNA kit, Omega Bio-Tek, Norcross, Georgia, USA) per manufacturer's instructions. Each sample was screened with PCR for the leukotoxin (*lktA*) gene with primers that amplified *lktA* in both *Mannheimia* species and *B. trehalosi* [9]. Positive samples were then analyzed using only the *Mannheimia lktA* gene PCR [7]. Samples positive on the initial PCR and negative on the second were categorized as *lktA* positive *B. trehalosi*. *Mannheimia* spp. *lktA* assay will amplify *lktA* in *M. haemolytica, M. glucosida,* and *M.ruminalis*. *Mannheimia haemolytica /glucosida lktA* specific PCR [70] was then performed on those samples positive for *Mannheimia lktA.*

*TSB.*A single tonsil swab was collected from animals as described in the Wyoming protocol and placed immediately into a vial of tryptic soy broth (TSB). Samples were frozen as soon as possible and shipped overnight on dry ice to Washington Animal Disease Diagnostic Laboratory (WADDL) for *Pasteurellaceae* culture following the lab's standard operating procedures. Swabs remained frozen at WADDL until they were plated by diagnosticians.

Port-A-Cul. A single tonsil swab was collected as described, placed in a Port-A-Cul ™ tube and kept chilled until received by the diagnostic lab. Samples were shipped overnight on ice packs to WADDL for *Pasteurellaceae* culture following the lab's

standard operating procedures. Samples were shipped to WADDL as soon as possible, arriving within 72 hours of collection. Samples were plated by WADDL immediately upon receipt.

Plated Culture. A single tonsil swab was collected as described and immediately used to inoculate a Columbia Blood Agar (CBA) culture plate with 5% sheep blood (Hardy Diagnostics, Santa Maria, California, USA) and a treated as described in the Wyoming PCR protocol. Following the Wyoming PCR protocol, the plate was struck to three quadrants for bacterial colony isolation the day of sample collection. After ~24 hours, a strip of the primary streak zone was swabbed with a sterile polyester-tipped swab, as were any phenotypically distinct colonies present on the plate. This swab was placed immediately into a vial of TSB. Samples were frozen immediately and shipped overnight on dry ice to WADDL for *Pasteurellaceae* culture. Swabs remained frozen at WADDL until they were plated by diagnosticians.

Plated PCR. Following completion of the MSU protocol, CBA plates were incubated an additional ~24 hours before bacterial growth was cleared from the CBA plate as described in the Wyoming PCR protocol. Samples were stored at approximately -20° C until being assessed by the Wyoming Game & Fish Department Wildlife Health Laboratory using the PCR procedures described in the Wyoming PCR protocol.

Mycoplasma ovipneumoniae

Wyoming. Samples from the nasal passages were obtained by inserting sterile polyester-tipped applicators (Puritan#25-806 1PD, Guilford, ME, USA) 8-12 cm into the nasal cavity and slowly rotating the shaft. The inoculated swab was then placed into transport media: Port-A-Cul™ tubes (Becton Dickinson, Franklin Lakes, NJ, USA) or 3ml Amies media without charcoal in a 15 x 103mm culture tube (Triforest Enterprises, Irvine, CA, USA). Swabs were removed from the transport media as previously described and placed into 2ml of modified tryptone soy broth (TSB-1) in sterile 5ml round-bottom tubes (BD Falcon, Franklin Lakes, NJ, USA) and incubated at 37°C with 5% CO_2 for 48 hours. DNA was extracted from 1 mL of the TSB-1 as described for the Wyoming PCR *Pasteurellaceae* protocol. DNA was analyzed using primers and PCR protocol published by McAuliffe [71], and optimized in the Wyoming Game and Fish Department Wildlife Health lab by modifying the initial denaturation for five minutes at 94°C, 32 denaturation cycles for 30 seconds each at 94°C, annealing at 57.5°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was at 72°C for 5 minutes. Samples were kept at 4°C until analyzed.

qPCR. A nasal swab was collected as described in the Wyoming PCR protocol and placed in a sterile cryovial without transport media and stored frozen. The samples were shipped to WADDL on dry ice and were tested for presence of *Mycoplasma ovipneumoniae* using quantitative PCR (qPCR) to aid in protocol development. This does not represent a FFS protocol.

TSB. A nasal swab was collected from animals sampled under this protocol and placed immediately into a vial of tryptic soy broth (TSB). Samples were frozen as soon as possible and shipped overnight on dry ice to Washington Animal Disease Diagnostic Laboratory (WADDL) for *Mycoplasma ovipneumoniae* PCR testing [39,71].

APPENDIX B

ASSESSING STABILITY OF DETECTION PROBABILITY ESTIMATES FOR BIGHORN SHEEP RESPIRATORY PATHOGENS.

Two subsets of data corresponding to samples obtained from bighorn sheep sampled for respiratory pathogens in Montana and bighorn sheep sampled in Wyoming were analyzed to assess stability and consistency of detection probability estimates for each *Pasteurellaceae* pathogen. The *Pasteurellaceae* TSB protocol was conducted on 456 of 476 individual bighorn sheep in the dataset, whereas other protocols were all conducted on less than 50% of the sampled individuals (Table B1). Therefore, if un-modeled heterogeneity in detection probability of the TSB Protocol existed, there was potential for bias in the estimated detection probability for the other protocols which were not conducted on the majority of individuals. Accordingly, the focus of this procedure was to estimate detection probability of each diagnostic protocol using only subsets of data where both that protocol and the TSB Protocol were conducted on all animals and compare the resulting estimates to that obtained from analysis of the complete dataset. The first subset (Montana) included only data from animals sampled in Montana, where samples from each animal were collected and tested using the Standard, MSU, and Plate-PCR protocols (n=152 individual animals). The Plate-PCR protocol did not assess presence of *Pasteurella multocida*, but this pathogen's detection probability using the Standard and MSU protocols was still assessed in this dataset. Detection probability for *Bibersteinia trehalosi* was not assessed in this dataset due insufficient detections (n=2). The second subset (Wyoming) included data from 122 bighorn sheep sampled in Wyoming, where samples were collected and tested using the WGF and Standard protocols.

The relative frequency with which the different *Mycoplasma ovipneumoniae* protocols were conducted follow the same pattern as with the *Pasteurellaceae* protocols; the Standard Protocol was conducted on 91% of the sampled animals, whereas the other protocols were conducted on <30% of the sampled animals (Table B2). Detection probability estimates from the two subsets of the complete *Mycoplasma ovipneumoniae* dataset, also corresponding to the state where the samples were collected (Montana or Wyoming), were obtained and compared those from the full dataset. The Montana subset included data from 106 animals sampled in Montana where two samples were assessed from each animal using the Standard protocol. The Wyoming subset included data from 95 animals sampled in Wyoming where samples were assessed using the Standard and Wyoming PCR protocols. The pathogen-specific subsets were then filtered to exclude population-years where that pathogen was not detected in order to minimize model convergence issues associated with estimating pathogen prevalence parameters at a boundary.

The same occupancy model structure was applied to all the datasets to assess consistency in the estimates of detection across the subsets of data. The occupancy model used for this assessment allowed each pathogen's estimated prevalence to vary by population-year and allowed each pathogen's estimated detection probability (ρ) to vary by diagnostic protocol. When detection probability estimates approached zero or one, boundary issues prevented the model from accurately estimating standard errors. In these cases, the standard errors were set to zero. This situation occurred when a protocol never detected a pathogen or when a protocol never failed to detect a pathogen in an animal

known to be infected. Fixing the standard errors was deemed more informative and accurate than using infinitely sized standard errors resulting from boundary issues.

For nearly every combination of pathogen and protocol, the parameter estimates for detection probability were similar and confidence intervals overlapped substantially (Figure B1); however detection probability estimates for the TSB protocol differed between the dataset for *Mannheimia* spp. (Figure B1). The detection probability estimate for this pathogen-protocol combination obtained from the complete dataset was 0.12 (95% CI: 0.08-0.16); the estimate from the Wyoming subset was 0.31 (95% CI: 0.21-0.43); and the estimate from the Montana subset was 0.01 (95% CI: 0.00-0.06). After confirming that the detection probability estimates for the other diagnostic protocols used to detect *Mannheimia* spp. were not strongly biased by this un-modeled heterogeneity in detection probability, the *Mannheimia* spp. detection probability estimates from the full dataset were kept based on the fact that there is no clear explanation for the heterogeneity of the TSB protocol and that the objectives of this paper require a single estimate of detection probability for each protocol.

Table B1. Number of animals sampled in each study population and year for *Pasteurellaceae* pathogens and the mean number of times each protocol was conducted per animal in parentheses. The total number of animals sampled and mean number of total diagnostic protocols conducted per animal are also shown.

Population-Year[1]	Standard	Classic	Plate PCR	MSU	Wyoming PCR	**TOTAL**
Castle Reef 14.15	23 (1)		21 (1)	16 (1)		23 (2.61)
Castle Reef 15.16	7 (2)		7 (1)	7 (2)		7 (5)
Clark Fork Cutoff 15.16*	4 (2)					4 (2)
Devil's Canyon 15.16	22 (1)				25 (1)	25 (1.88)
Dubois Badlands 15.16	4 (1)				5 (1)	5 (1.8)
Mt Everts 13.14	5 (1)					5 (1)
Fergus 14.15	60 (1)		29 (1)	15 (1)		60 (1.73)
Franc's Peak 12.13*	2 (1)				2 (1)	2 (2)
Franc's Peak 14.15*	3 (1)			3 (1)	3 (1)	3 (3)
Franc's Peak 15.16*	1 (1)				2 (1)	2 (1.5)
Highlands 15.16	16 (2)					16 (2)
Hilgard 13.14	29 (1)					29 (1)
Hilgard 14.15	49 (1.98)		38 (1)	18 (1)		49 (3.12)
Hilgard 15.16	34 (1.97)	34 (1)	34 (1)	31 (1.97)		34 (5.77)
Jackson 12.13*	1 (1)				3 (1)	3 (1.33)
Jackson 15.16	16 (1)				16 (1)	16 (2)
Lost Creek 14.15	13 (1)		13 (1)	13 (1)		13 (3)
Lost Creek 15.16	6 (2)		5 (1)	6 (2)		6 (4.83)
Middle Missouri Breaks 15.16	19 (2)					19 (2)
N. Clark Fork 15.16*	1 (2)		1 (1)	1 (2)		1 (5)
Perma-Paradise 14.15	30 (1)		28 (1)	15 (1)		30 (2.43)
Petty Creek 15.16	16 (2)	17 (1)	17 (1)	17 (2)		17 (5.88)
Stillwater 14.15	16 (1)		16 (1)	16 (1)		16 (3)
Stillwater 15.16*	3 (2)		3 (1)			3 (3)
Sybille 15.16	11 (2)	11 (1)		11 (1)	11 (1)	11 (5)
Temple Peak 15.16	9 (1)				14 (1)	14 (1.64)
Trout Peak 12.13*	1 (1)				1 (1)	1 (2)
Trout Peak 14.15*	1 (2)					1 (2)
Trout Peak 15.16	7 (1)				8 (1)	8 (1.88)
Wapiti Ridge 12.13	16 (1)				16 (1)	16 (2)
Wapiti Ridge 14.15	7 (1)			7 (1)	7 (1)	7 (3)
Wapiti Ridge 15.16	10 (1)				15 (1)	15 (1.67)

Population-Year[1]	Standard	Classic	Plate PCR	MSU	Wyoming PCR	**TOTAL**
Castle Reef 14.15	23 (1)		21 (1)	16 (1)		23 (2.61)
Castle Reef 15.16	7 (2)		7 (1)	7 (2)		7 (5)
Whiskey Mountain 15.16	12 (1)				8 (1)	13 (1.54)
NE Yellowstone 14.15*	2 (1)					2 (1)
TOTAL	456 (1.36)	62 (1)	212 (1)	176 (1.34)	136 (1)	**476 (2.66)**

[1] Population –Years where less than five individual bighorn sheep were sampled were not considered in analysis and are denoted with an asterisk (*).

Table B2. Number of animals sampled in each study population and year for *Mycoplasma ovipneumoniae* and the mean number of times each protocol was conducted per animal in parentheses. The total number of animals sampled and mean number of total diagnostic protocols conducted per animal are also shown.

Population-Year[1]	Standard	qPCR	Wyoming PCR	**TOTAL**
Castle Reef 14.15	23 (1)	15 (1)		23 (1.65)
Castle Reef 15.16	7 (2)			7 (2)
Clark Fork Cutoff 15.16*	4 (2)			4 (2)
Devil's Canyon 15.16			25 (1)	25 (1)
Dubois Badlands 15.16	5 (1)		5 (1)	5 (2)
Mt Everts 13.14	5 (1)			5 (1)
Fergus 14.15	59 (1)	29 (1)		59 (1.49)
Franc's Peak 12.13*	2 (1)		2 (1)	2 (2)
Franc's Peak 14.15*			3 (1)	3 (1)
Franc's Peak 15.16*	2 (1)		2 (1)	2 (2)
Highlands 15.16	16 (2)			16 (2)
Hilgard 13.14	29 (1)			29 (1)
Hilgard 14.15	50 (1)			50 (1)
Hilgard 15.16	35 (2)			35 (2)
Jackson 15.16	16 (1)		16 (1)	16 (2)
Lost Creek 14.15	13 (1)	7 (1)		13 (1.54)
Lost Creek 15.16	6 (2)			6 (2)
Middle Missouri Breaks 15.16	19 (2)			19 (2)
N. Clark Fork 15.16*	1 (1)			1 (1)
Paradise 14.15	30 (1)	30 (1)		30 (2)
Petty Creek 15.16	16 (2)			16 (2)
Stillwater 14.15	16 (1)	7 (1)		16 (1.44)
Stillwater 15.16*	3 (2)			3 (2)
Sybille 15.16	11 (2)		11 (1)	11 (3)
Temple Peak 15.16	12 (1)		14 (1)	14 (1.86)
Trout Peak 12.13*	1 (1)		1 (1)	1 (2)
Trout Peak 14.15*	2 (1)			2 (1)
Trout Peak 15.16	8 (1)		8 (1)	8 (2)
Wapiti Ridge 12.13	16 (1)		16 (1)	16 (2)
Wapiti Ridge 14.15			7 (1)	7 (2)
Wapiti Ridge 15.16	14 (1)		15 (1)	15 (1.93)
Whiskey Mountain 15.16	8 (1)		8 (1)	8 (2)
NE Yellowstone 14.15*	2 (1)			2 (1)
TOTAL	431 (1.27)	88 (1)	133 (1)	**469 (1.64)**

[1] Population –Years where less than five individual bighorn sheep were sampled were not considered in analysis and are denoted with an asterisk (*).

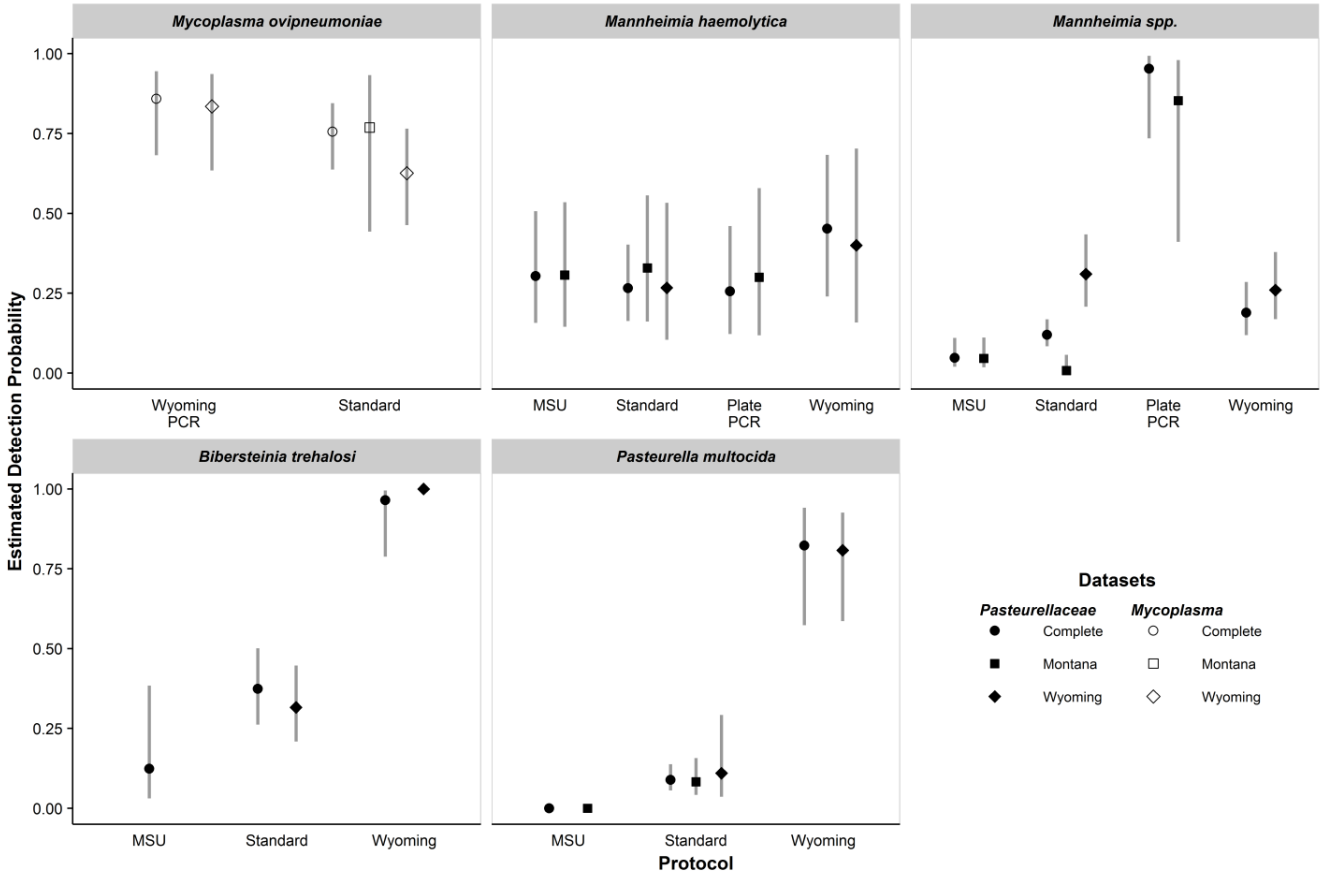


Figure B1. Estimated detection probabilities and 95% confidence intervals of the respiratory pathogens obtained from independent analyses of the complete dataset and two subsets considering only data from bighorn sheep where multiple protocols were conducted. The subsets are labeled to denote the state where the data within them were collected. The different diagnostic protocols are shown on the x-axis of each facet and the detection probability estimates obtained for each protocol from analysis of the different datasets are shown adjacent to each other in different shapes. The occupancy model structure used to obtain these estimates allowed pathogen prevalence to vary by bighorn population and detection probability to vary by protocol.

APPENDIX C

DERIVATION OF DETECTION POWER SOLUTION

The estimates of detection probability for a single sample were scaled to the population level, while considering the effect of collecting multiple samples per individual animal. The joint probability of obtaining a positive test from a single sample from an individual animal, given the animal is infected and assuming perfect specificity (i.e., no false positive tests) can be expressed as $\Pr(X_{ij}^{+}, \rho_{ij}) = \Pr(X_{ij}^{+}|\rho_{ij}) \times \Pr(\rho_{ij})$, where X_{ij}^{+} indicates the event of a positive test result for the j^{th} pathogen using the i^{th} protocol, and ρ_{ij} indicates the corresponding probability of obtaining a positive test, given an animal is infected (i.e. detection probability). This joint probability was factored into the following conditional probability statement:

$$\Pr(X_{ij}^{+}, \rho_{ij}) = \rho \frac{\Gamma(a_{ij}+\beta_{ij})}{\Gamma a_{ij}\Gamma\beta_{ij}} \rho_{ij}{}^{\alpha-1}(1-\rho_{ij})^{\beta_{ij}-1},$$

where α and β conceptually represent the number of positive and negative tests, respectively, from which ρ_{ij} was estimated. The conditional probability statement was marginalized over ρ to obtain the following expression:

$$\Pr(X_{ij}^{+}) = \int_0^1 \rho_{ij} \frac{\Gamma(a_{ij}+\beta_{ij})}{\Gamma a_{ij}\Gamma\beta_{ij}} \rho^{\alpha-1}(1-\rho_{ij})^{\beta-1}\ d\rho_{ij}$$

Because the kernel of the integral represents a beta distribution, the expression could be simplified to show the probability of obtaining a positive test result in a single sample collected from an infected animal is: $\Pr(X_{ij}^{+}) = a_{ij}/(a_{ij}+\beta_{ij})$, where α_{ij} and β_{ij} describe

the beta distribution for the detection probability of the i^{th} diagnostic protocol used to test a given sample for presence of the j^{th} pathogen.

The estimates of detection probability for each pathogen-protocol combination were incorporated into this expression by first creating a logit-normal distribution using the detection parameter estimate and associated standard error as the mean and standard deviation of the distribution. The distribution was then transformed to the probability scale and used maximum-likelihood estimation via the "fitdistrplus" package [72] in Program R to redefine it as a beta distribution that could be used to solve the equation. Any detection probabilities that were estimated on a boundary were not incorporated into the subsequent detection probability analysis. Comparison of the quantiles of the model-estimated detection probability distributions and the corresponding derived beta distributions confirmed the corresponding distributions matched each other well (Table C1).

The probability of obtaining a positive test result from each sample assessed (using the i^{th} protocol to detect the j^{th} pathogen) from an infected animal can be described by the following Bernoulli distribution: $X_{ij}^{+} \sim Bernoulli\ (a_{ij}/(a_{ij} + \beta_{ij}))$. The complementary probability of obtaining a negative test result in the same sample is described by: $X_{ij}^{-} \sim Bernoulli\ (1 - a_{ij}/(a_{ij} + \beta_{ij}))$, where X_{ij}^{-} indicates the event of negative test result. Assuming independence among replicate assessments of pathogen presence for the same animal, the sum of each replicate assessment of pathogen presence (using the same protocol) amounts to the binomial distribution:
$\Pr(T_{ij} \sim Binomial\ (\#samples, 1 - a_{ij}/(a_{ij} + \beta_{ij}))$, where T_{ij} indicates the number of

negative test results obtained from the replicate sampling. The probability of obtaining negative tests across all replicate trials is then described as:$\Pr(T_{ij}^{-}) = (1 - a_{ij}/(a_{ij} + \beta_{ij}))^{s}$, where T_{ij}^{-} indicates the event of all replicate trials (assessing presence of the j^{th} pathogen using the i^{th} protocol) returning a negative result and *s* represents the number of replicate trials. The complementary probability of obtaining at least one positive is: $\Pr(T_{ij}^{+}) = 1 - (1 - a_{ij}/(a_{ij} + \beta_{ij}))^{s}$, where T^{+}_{ij} indicates the outcome of at least one replicate trial returning a positive result. This formula was used to scale the results to the population level.

The power to detect pathogen *j* in sampled population *k* depends on the probability of detecting pathogen *j* in each infected animal as well as the number of infected animals (I_k) that are sampled, the latter of which follows a hypergeometric distribution[73]. The joint probability of *not* detecting the j^{th} pathogen in population *k* while sampling *I* infected animals using the i^{th} protocol was calculated as:

$$\Pr(I_{jk}, N_{ij}^{-}) =$$

$$\Pr(N_{ij}^{-} | I_{jk}) \times \Pr(I_{jk}) =$$

$$\left[\left(1 - \frac{a_{ij}}{a_{ij} + \beta_{ij}}\right)^{s}\right]^{I_k} \times \frac{\binom{N_k \times \psi_{jk}}{I_{jk}}\binom{N_k - N_k \times \psi_{jk}}{n_k - I_{jk}}}{\binom{N_k}{n_k}}$$

where N_k equals the number of animal in population *k*, ψ_{jk} equals the prevalence of pathogen *j* in population *k*, $N_k \times \psi_{jk}$ equals the number of animals infected by pathogen *j* in population *k*, and n_k equals the number of animals sampled in population *k*. When $N_k \times \psi_{jk}$ was not an integer, the value was down to the next integer. This probability statement was marginalized over I_{jk} to provide the following solution for the probability of not detecting pathogen *j* after sampling *n* animals from population *k* using protocol *i*.

$$\Pr\left(N_{ij}^{-}\right) = \sum_{I_{jk}=0}^{\min(n_k, N_k\psi_{jk})} \left[\left(1 - \frac{a_{ij}}{a_{ij} + \beta_{ij}}\right)^{s}\right]^{I_k} \times \frac{\binom{N_k \times \psi_{jk}}{I_{jk}}\binom{N_k - N_k \times \psi_{jk}}{n_k - I_{jk}}}{\binom{N_k}{n_k}}$$

Accordingly, the complementary probability of detecting pathogen *j* in at least one sample $\left(N_{ij}^{+}\right)$ after sampling *n* animals from population *k* using protocol *i* can be calculated as:

$$\Pr\left(N_{ij}^{+}\right) = 1 - \sum_{I_{jk}=0}^{\min(n_k, N_k\psi_{jk})} \left[\left(1 - \frac{a_{ij}}{a_{ij} + \beta_{ij}}\right)^{s}\right]^{I_k} \times \frac{\binom{N_k \times \psi_{jk}}{I_{jk}}\binom{N_k - N_k \times \psi_{jk}}{n_k - I_{jk}}}{\binom{N_k}{n_k}}$$

Table C1. Distribution parameters and quantiles obtained directly from model estimates of detection probability (Logit-Normal) for each pathogen-protocol combination (not estimated on a boundary) and the corresponding derived beta distributions that were used to assess detection power.

Pathogen	Protocol	Distribution Parameters		25% Quantile		50% Quantile		75% Quantile	
		Logit-Normal (μ,σ^2)	*Beta* (α, β)	Logit-Normal	Beta	Logit-Normal	Beta	Logit-Normal	Beta
Mannheimia haemolytica									
	Plated PCR	-1.07, 0.46	6.63, 18.36	0.201	0.202	0.256	0.259	0.319	0.321
	Port-A-Cul	-2.18, 1.07	1.22, 7.34	0.052	0.055	0.101	0.115	0.189	0.203
	Plated Culture	-0.83, 0.44	8, 17.65	0.246	0.247	0.304	0.307	0.370	0.371
	Standard	-1.02, 0.32	14.01, 37.82	0.226	0.227	0.266	0.267	0.310	0.310
	Wyoming	-0.19, 0.49	8.08, 9.71	0.372	0.373	0.452	0.452	0.534	0.533
Mannheimia spp.									
	Plated PCR	3.01, 1.02	16.11, 1.22	0.911	0.901	0.953	0.946	0.976	0.974
	Plated Culture	-2.99, 0.46	5.13, 93.05	0.035	0.036	0.048	0.049	0.064	0.065
	TSB	-2, 0.2	27.96, 202.63	0.106	0.106	0.120	0.120	0.135	0.135
	Wyoming	-1.46, 0.28	16.66, 69.99	0.162	0.163	0.189	0.190	0.219	0.219
Bibersteinia trehalosi									
	Plated Culture	-2.15, 0.75	2.26, 15.79	0.066	0.068	0.105	0.112	0.162	0.168
	TSB	-0.59, 0.26	23.28, 41.64	0.317	0.318	0.356	0.357	0.398	0.398
	Wyoming	3.29, 1.02	20.42, 1.2	0.931	0.923	0.964	0.958	0.982	0.980
Pasteurella multocida									
	Port-A-Cul	-0.25, 1.29	1.48, 1.77	0.246	0.258	0.437	0.444	0.650	0.642
	TSB	-1.9, 0.26	16.84, 110.23	0.111	0.111	0.130	0.131	0.151	0.152
	Wyoming	1.58, 0.63	14.48, 3.36	0.761	0.756	0.829	0.823	0.882	0.879
Mycoplasma ovipneumoniae									
	qPCR	0.59, 0.69	6.28, 3.69	0.531	0.530	0.643	0.638	0.742	0.738
	TSB	0.98, 0.25	60.18, 22.91	0.693	0.692	0.727	0.726	0.759	0.758
	Wyoming	1.85, 0.53	25, 4.36	0.815	0.812	0.864	0.859	0.901	0.899

APPENDIX D

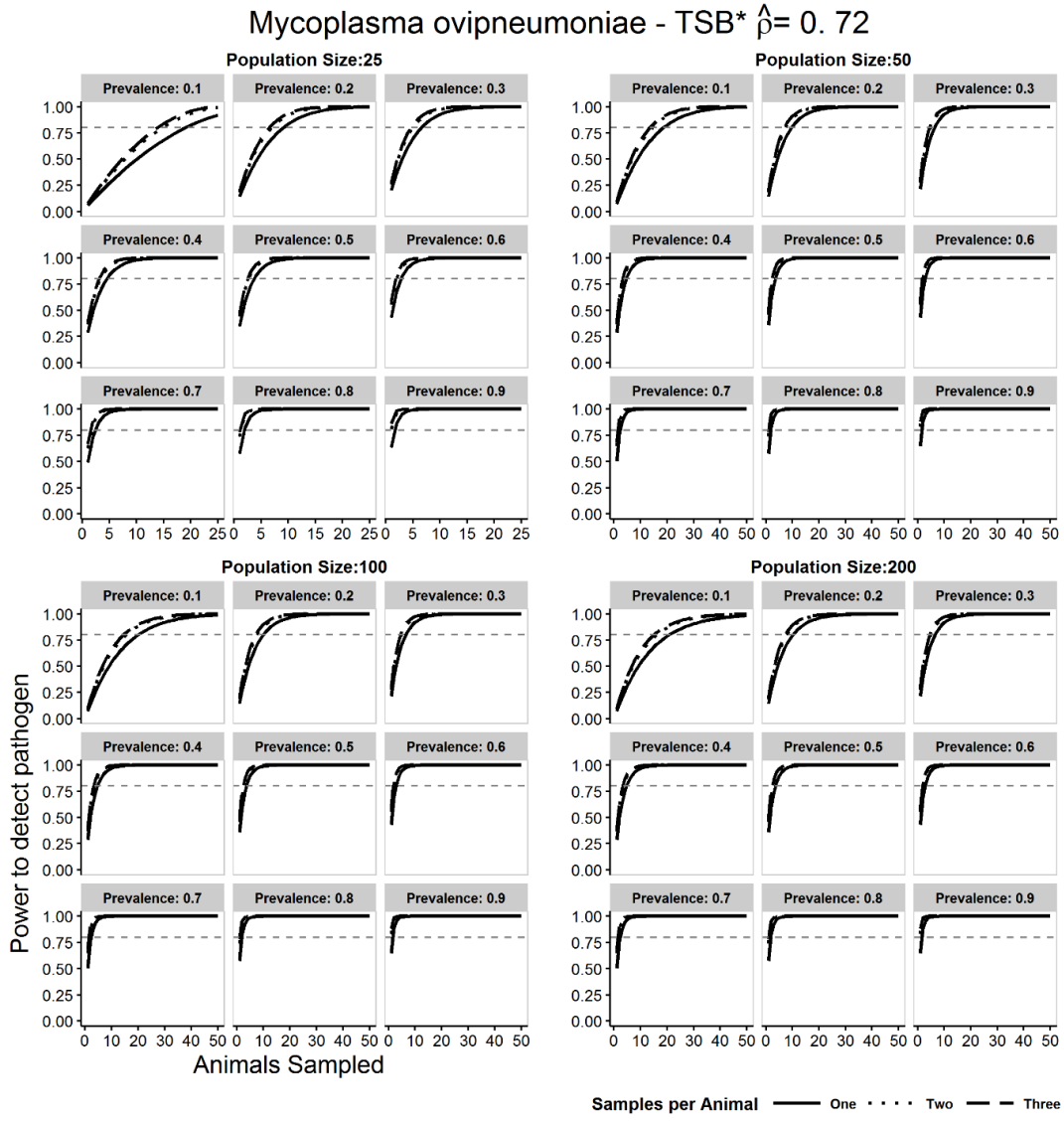
DETECTION POWER CURVES FOR ALL PATHOGEN-PROTOCOL COMBINATIONS

These charts are intended to provide readers the ability to investigate suitable sampling methodologies and intensities based on their capabilities, restrictions, and preferences. Each page in this appendix illustrates the effects of various factors on the power to detect a pathogen in a sampled population, given the pathogen and the protocol used, which is stated at the top of the graphic, along with the estimated detection probability for that pathogen-protocol combination. Protocols that use a fee-for-service (FFS) diagnostic test are indicated with an asterisk following their name. Each page is split into quadrants to illustrate relationships under four different population sizes (N=25, 50, 100, & 200). Each quadrant is split into nine panels to show expected relationships through a sequence of pathogen prevalence ranging from 0.1 to 0.9. Each panel shows the power to detect the pathogen (y-axis), given the number of animals sampled (x-axis) and three lines are used to show this relationship when protocol are conducted one, two, and three times per animal. The horizontal dashed line in each panels corresponds with 80% power to detect the pathogen. The x-axes in the quadrant illustrating these relationships for a population size of 25 differs from the x-axes of the other quadrants. Power curves for several pathogen-protocol combination are not shown in the appendix because the protocol never successfully detected that particular pathogen. Pages are organized by pathogen family (*Pasteurellaceae* or *Mycoplasma*) and then by Protocols.

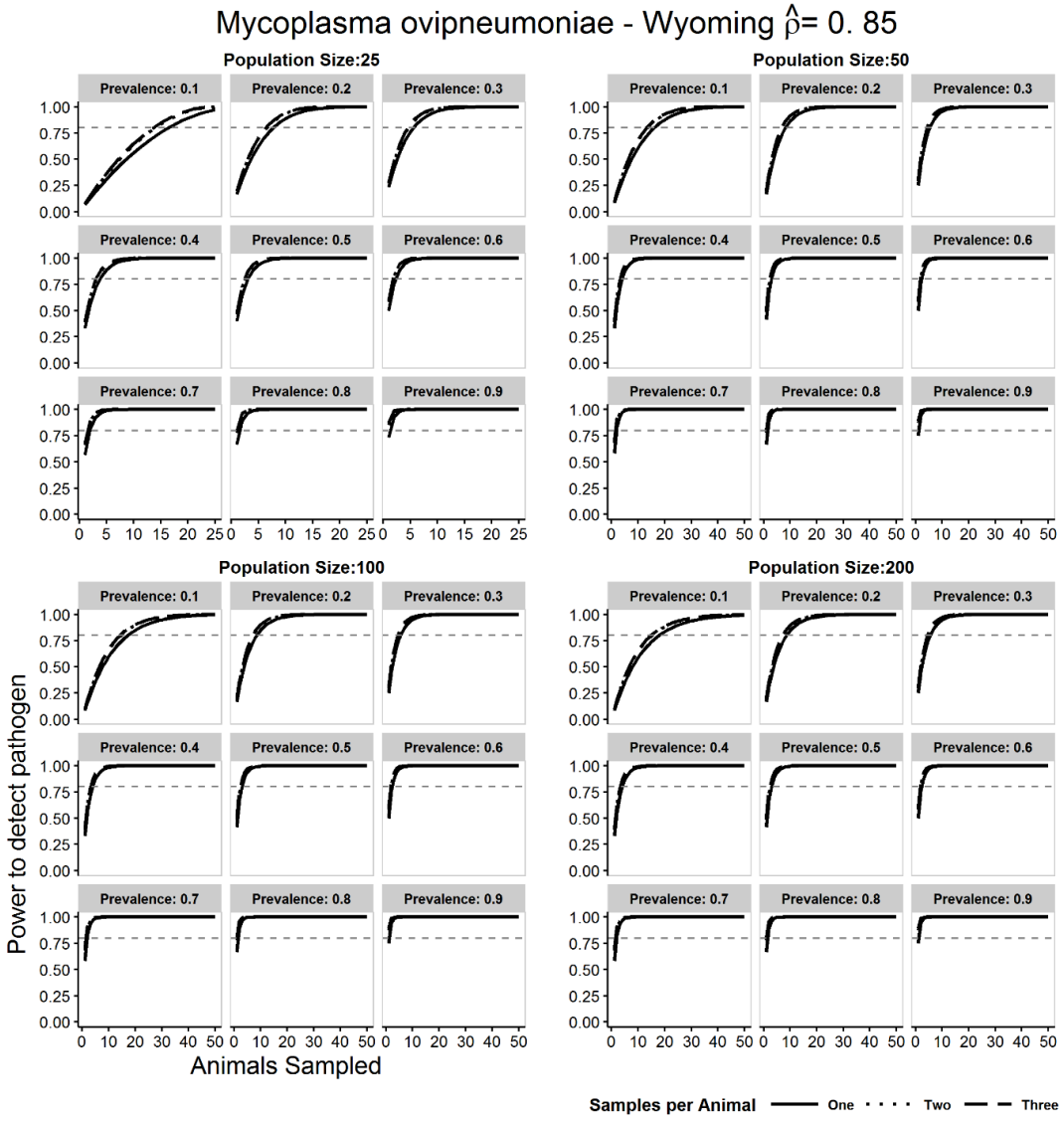
Table of Contents

Mycoplasma ovipneumoniae

TSB*

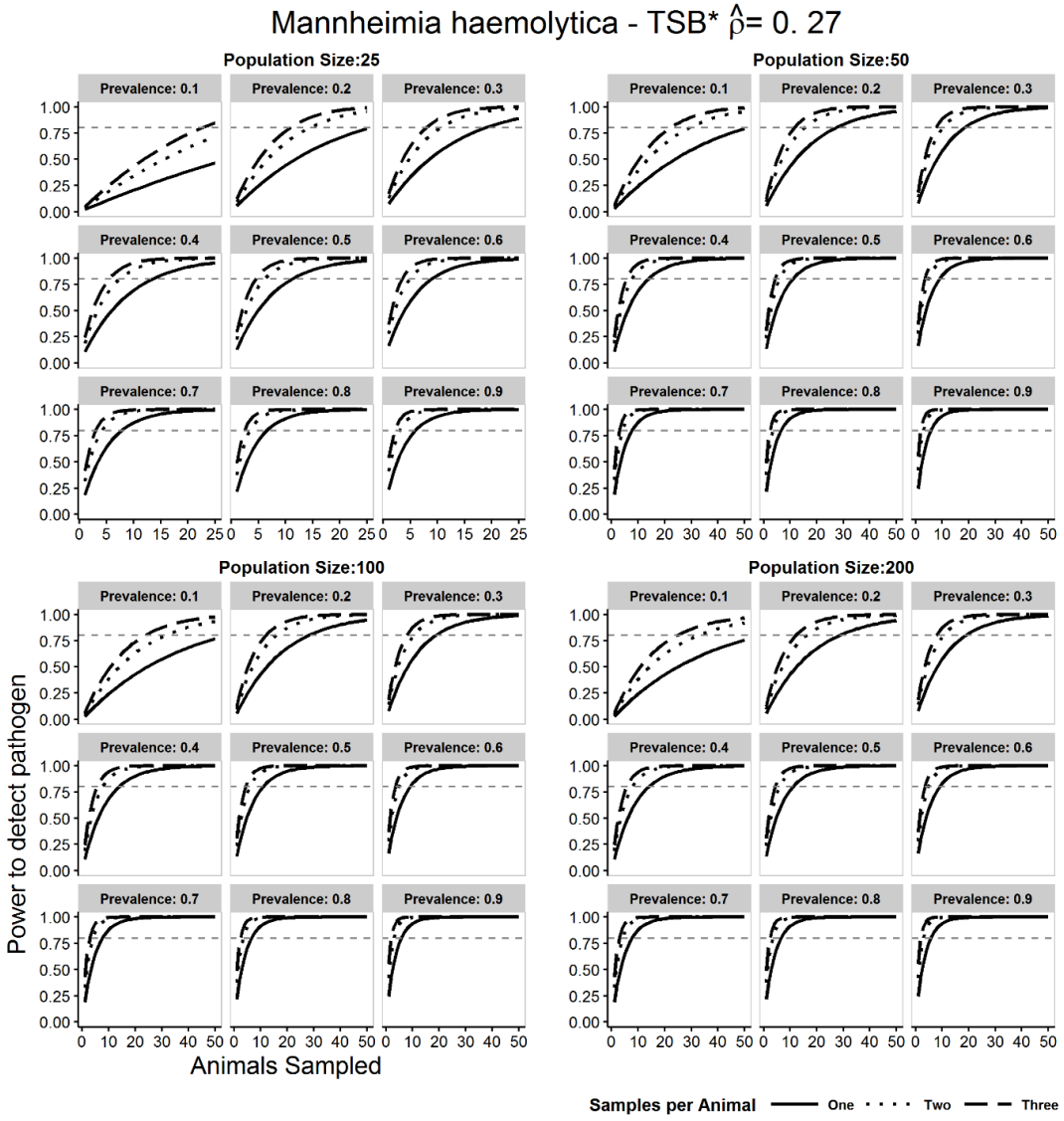


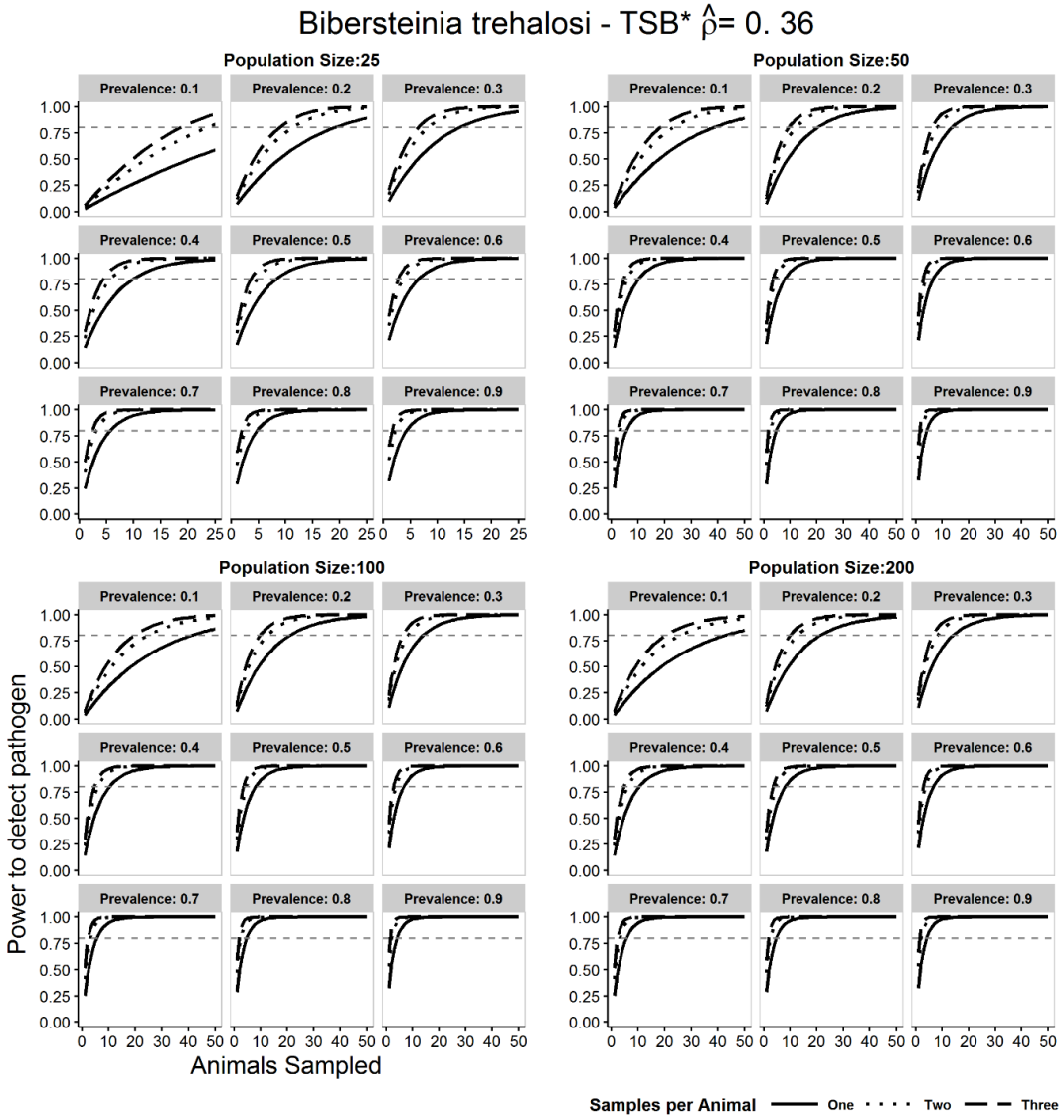
Wyoming

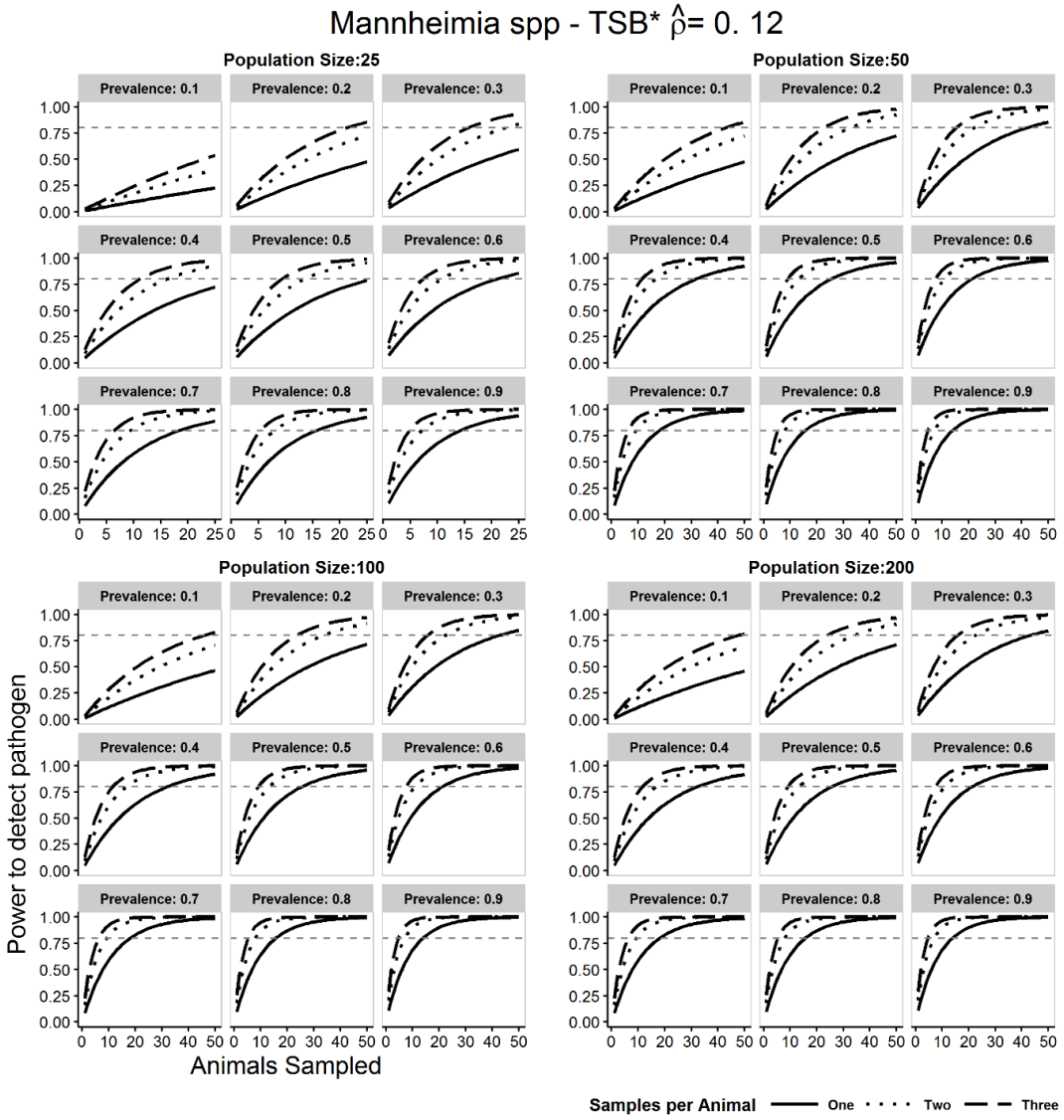

Mycoplasma ovipneumoniae - Wyoming $\hat{\rho}$= 0. 85
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

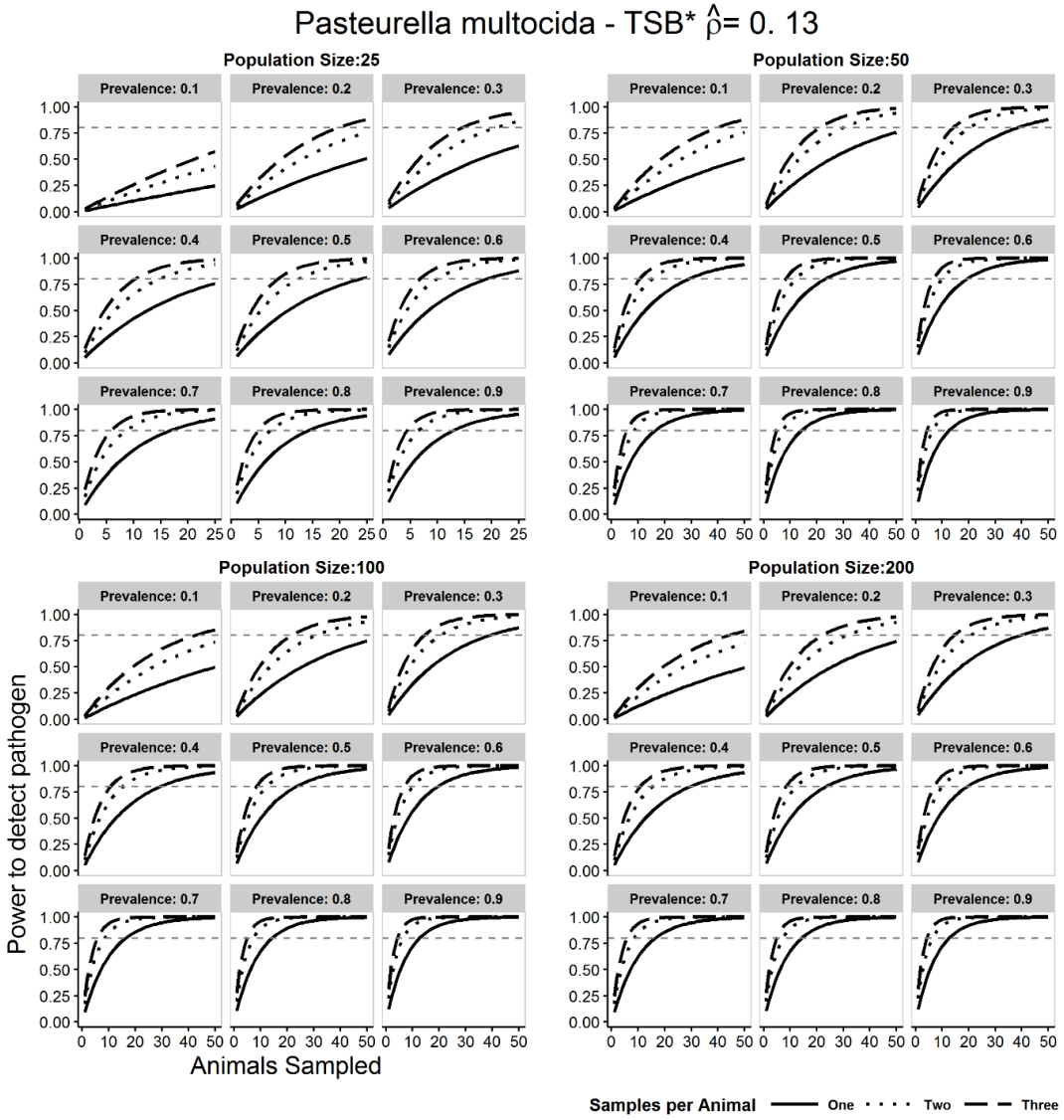
Pasteurellaceae

TSB*

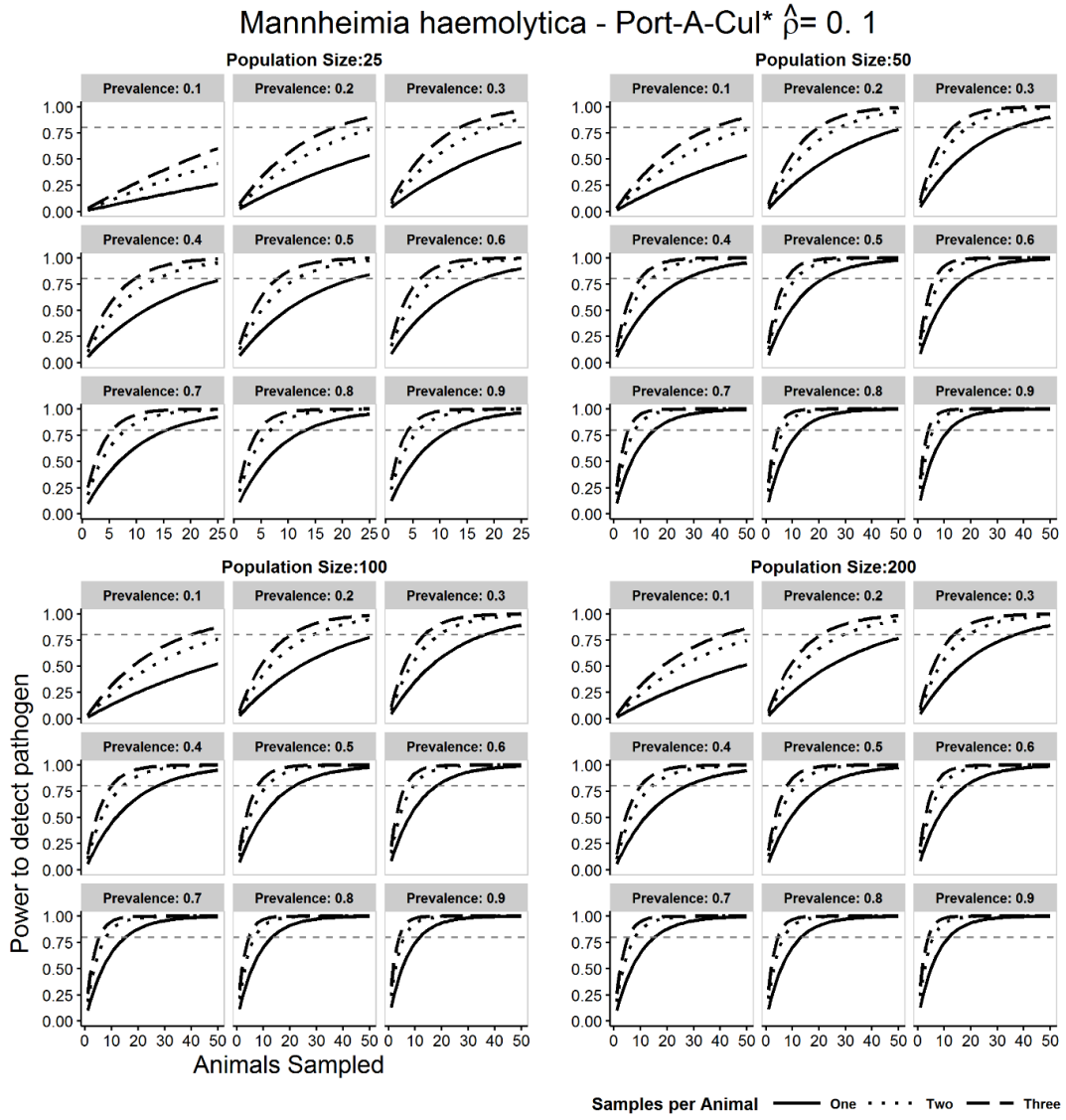


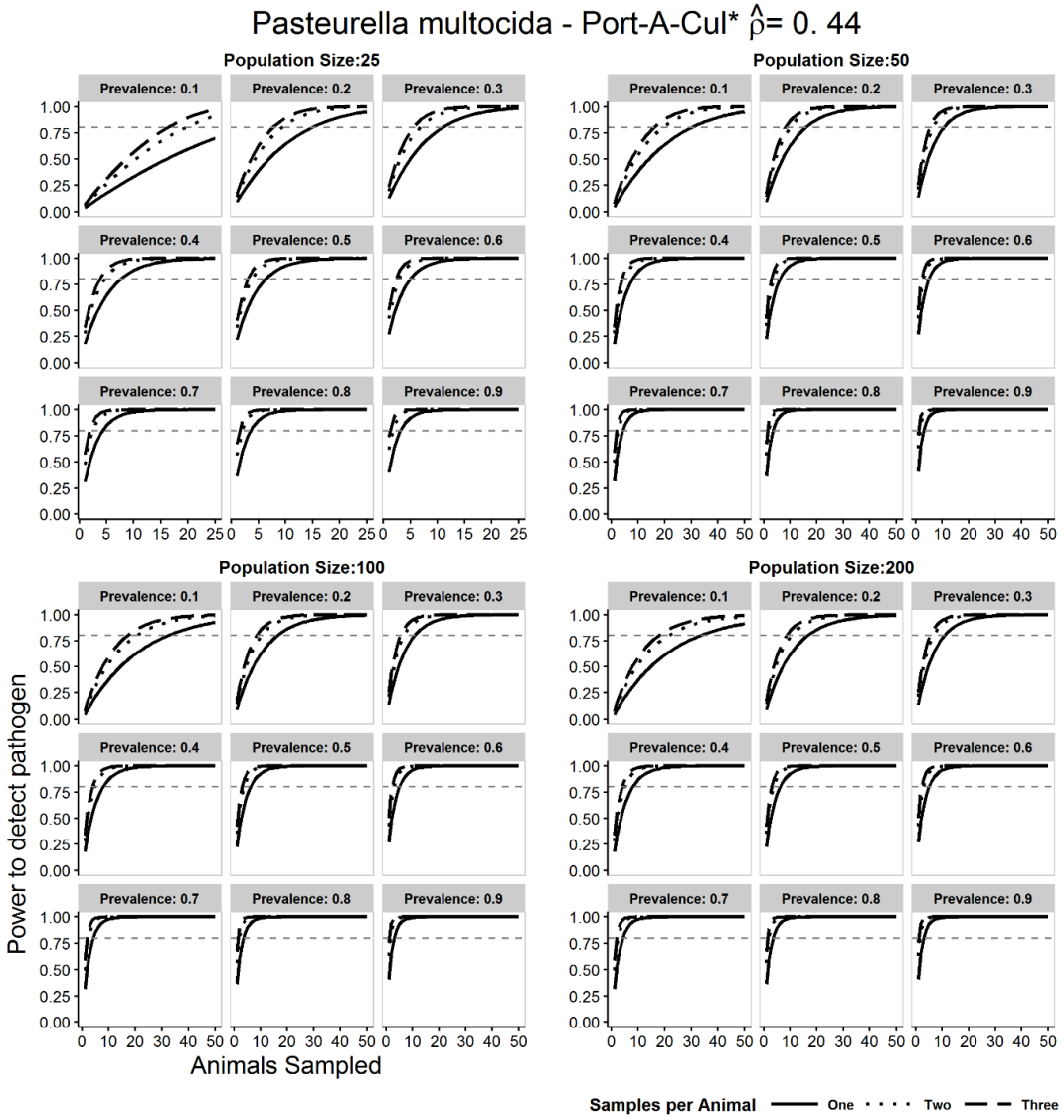

Bibersteinia trehalosi - TSB* $\hat{\rho}$= 0. 36
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three


Mannheimia spp - TSB* ρ̂= 0. 12
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

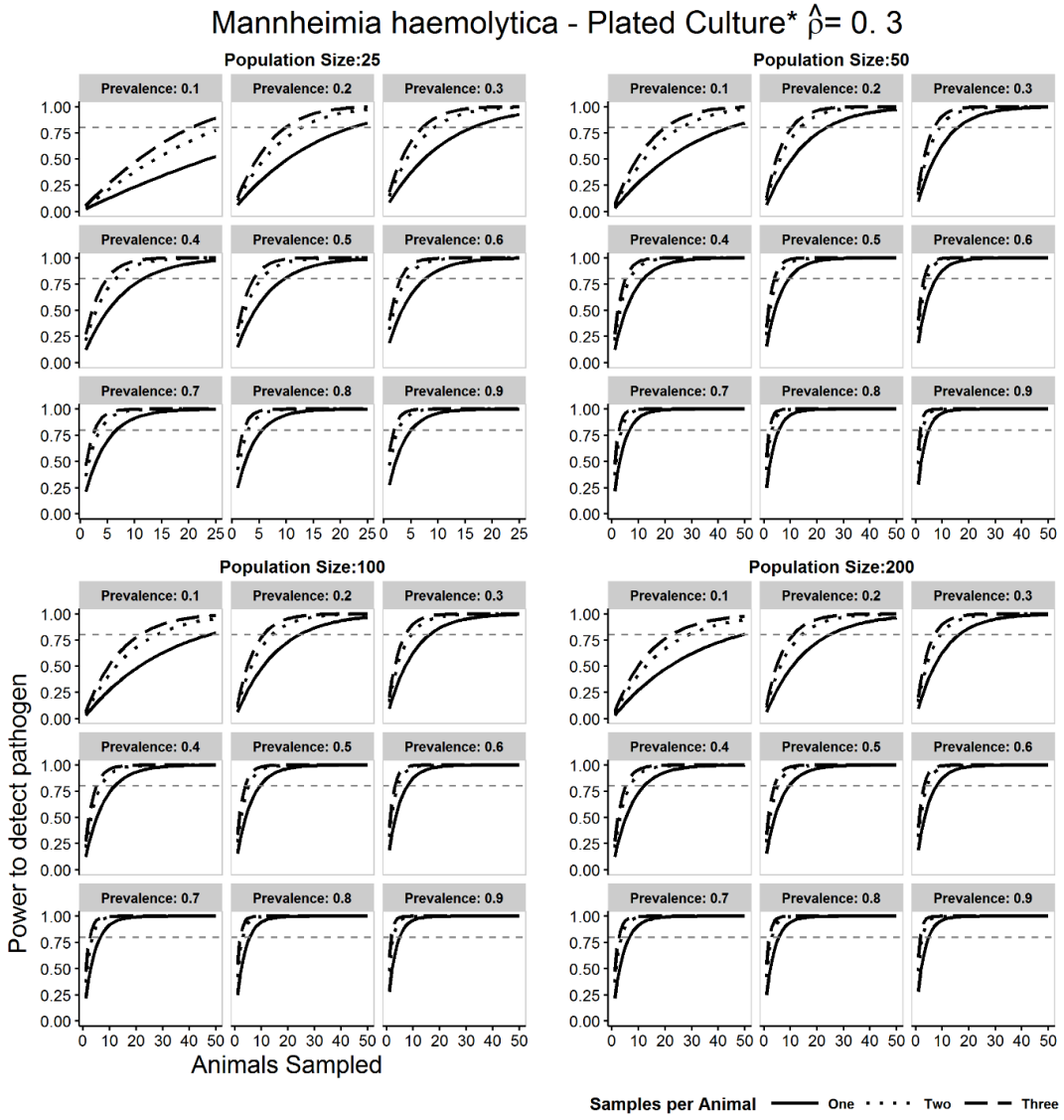

Pasteurella multocida - TSB* $\hat{\rho}$= 0. 13
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

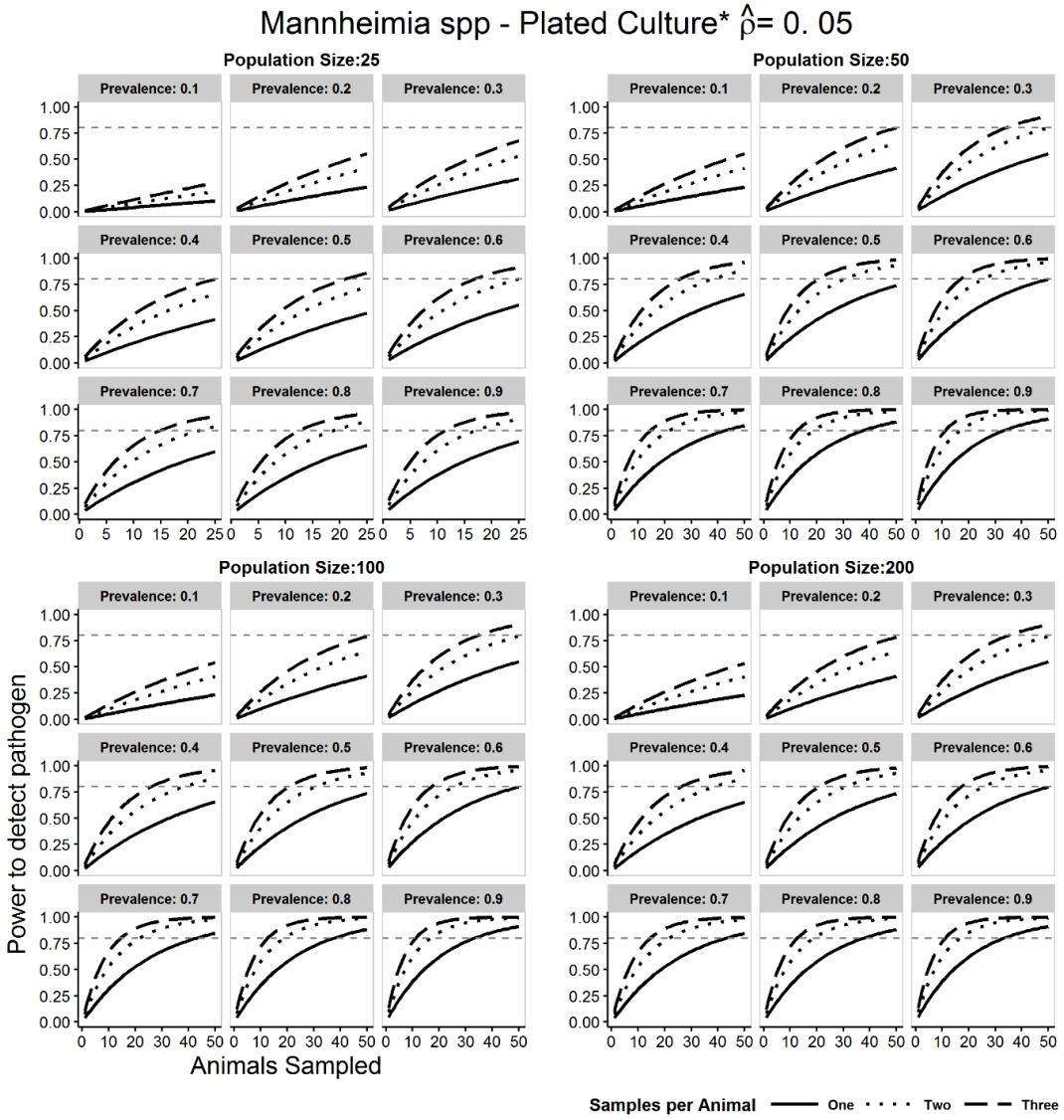
Port-A-Cul*

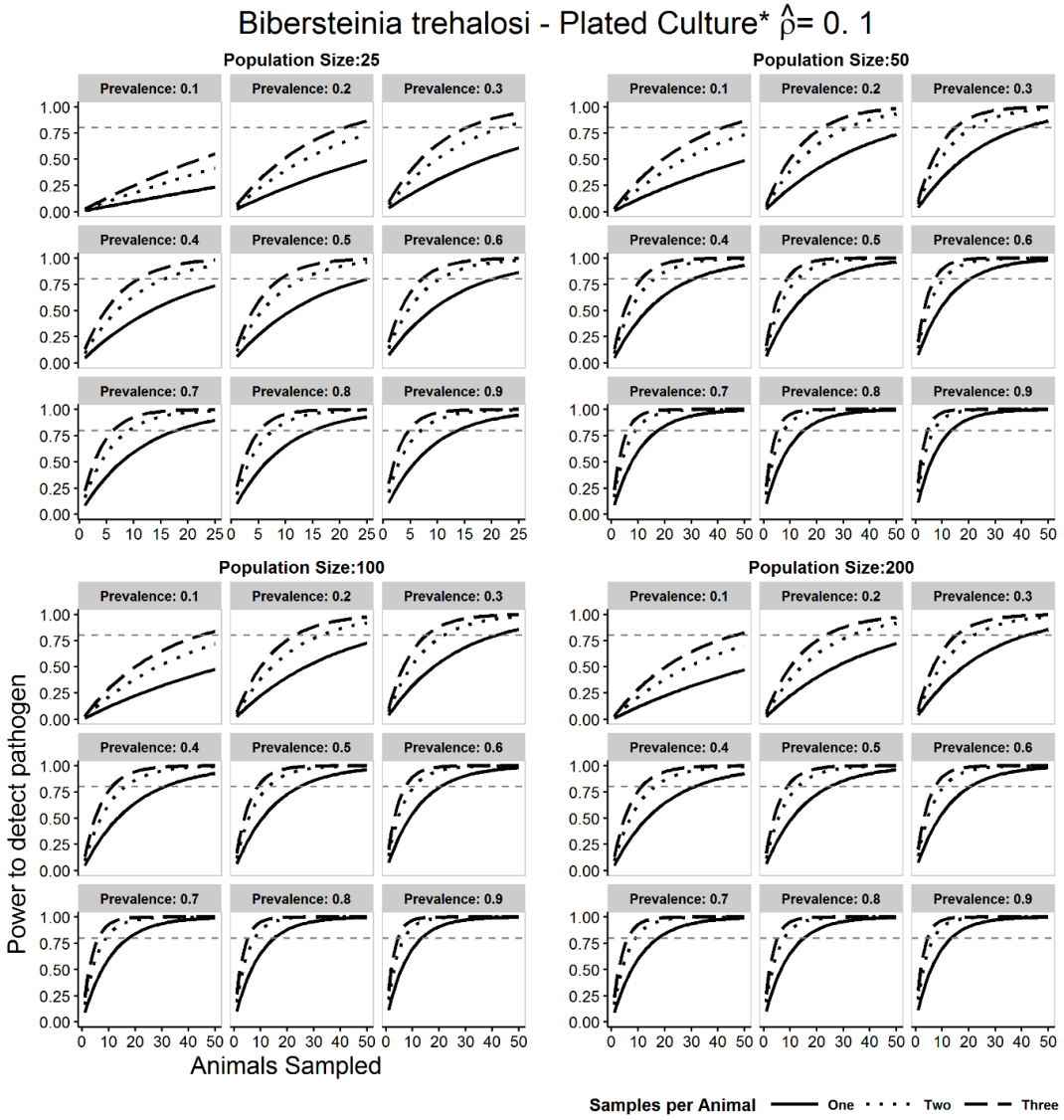

Mannheimia haemolytica - Port-A-Cul* ρ̂= 0. 1
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three


Pasteurella multocida - Port-A-Cul* $\hat{\rho}$= 0. 44
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

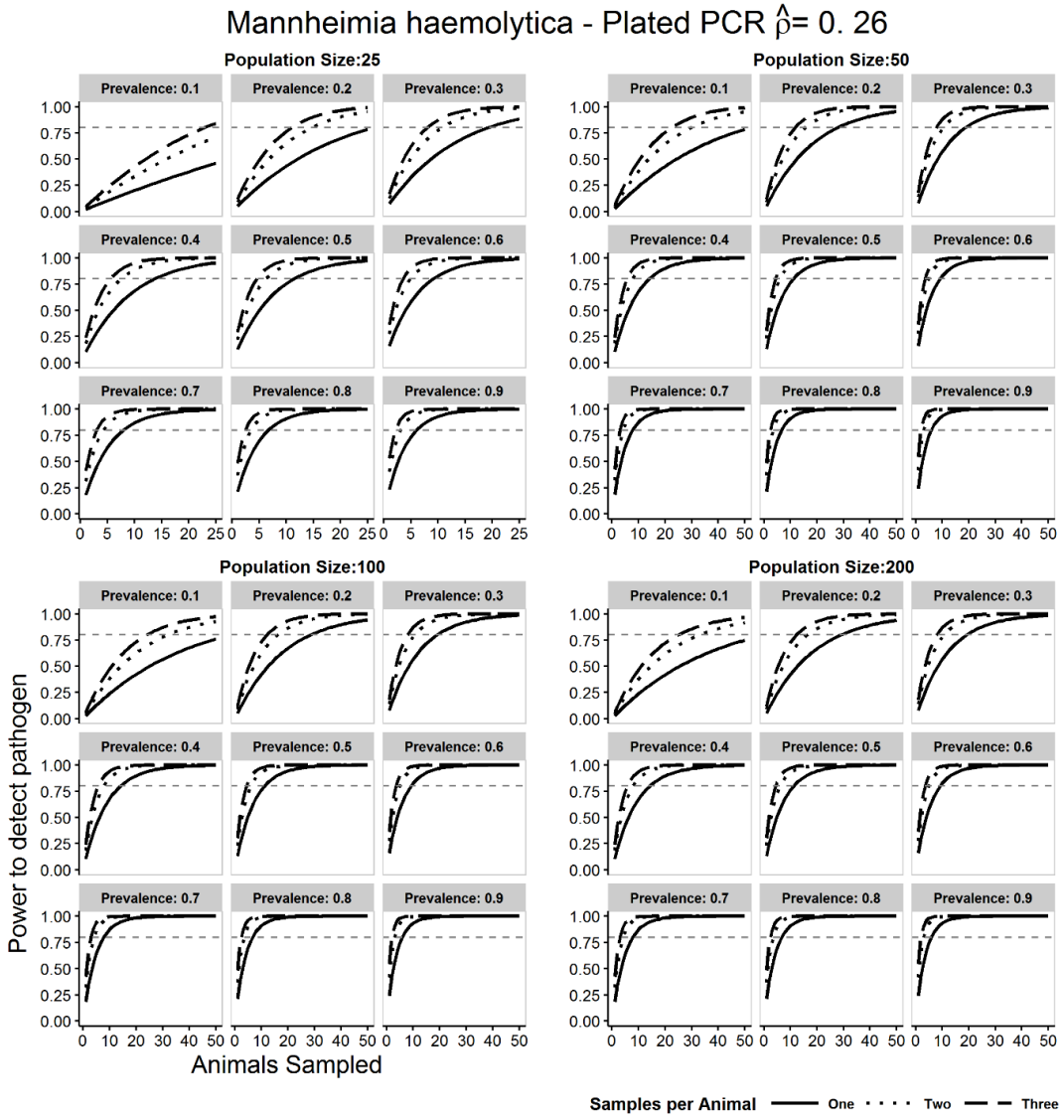
Plated Culture*

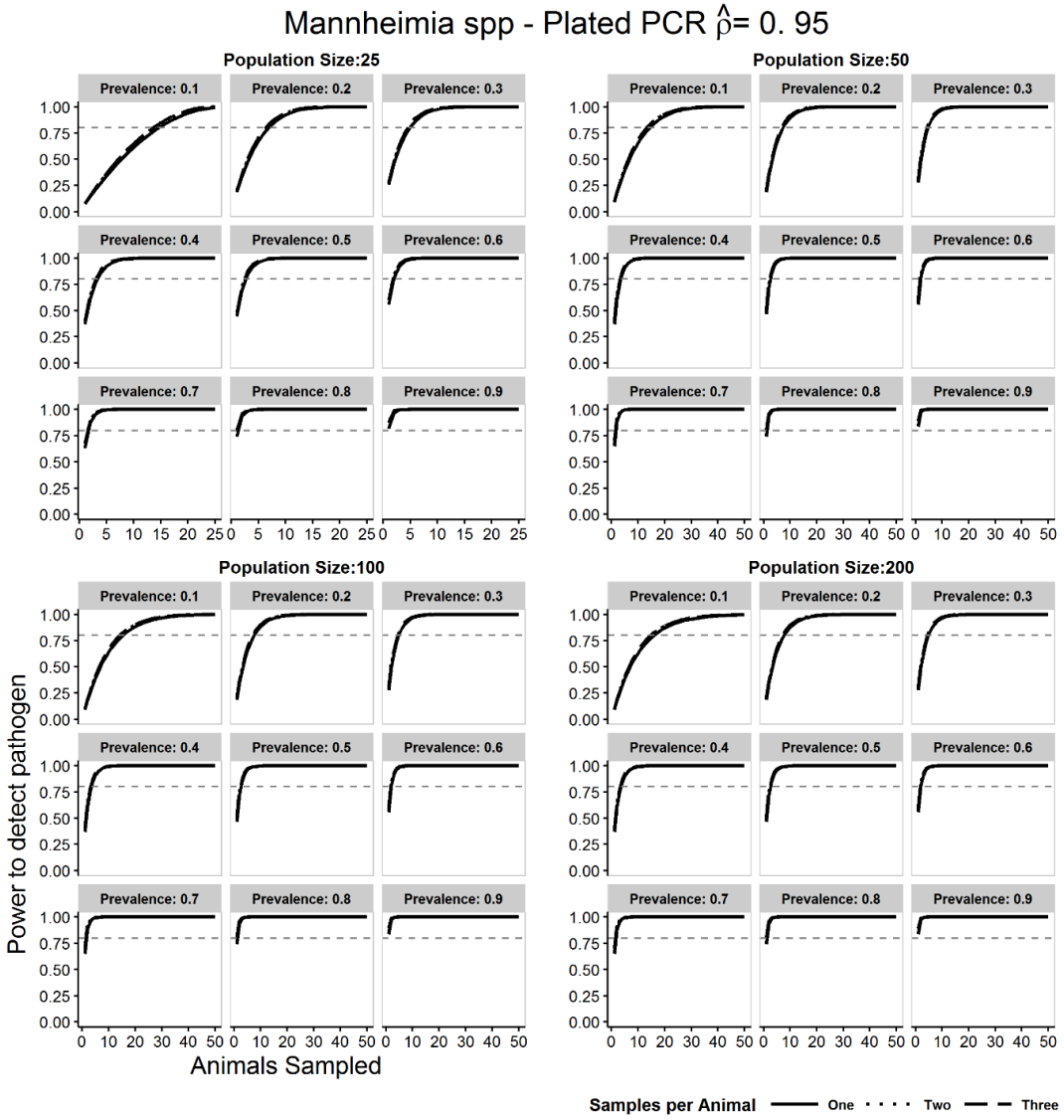



Mannheimia spp - Plated Culture* $\hat{\rho}$= 0. 05
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

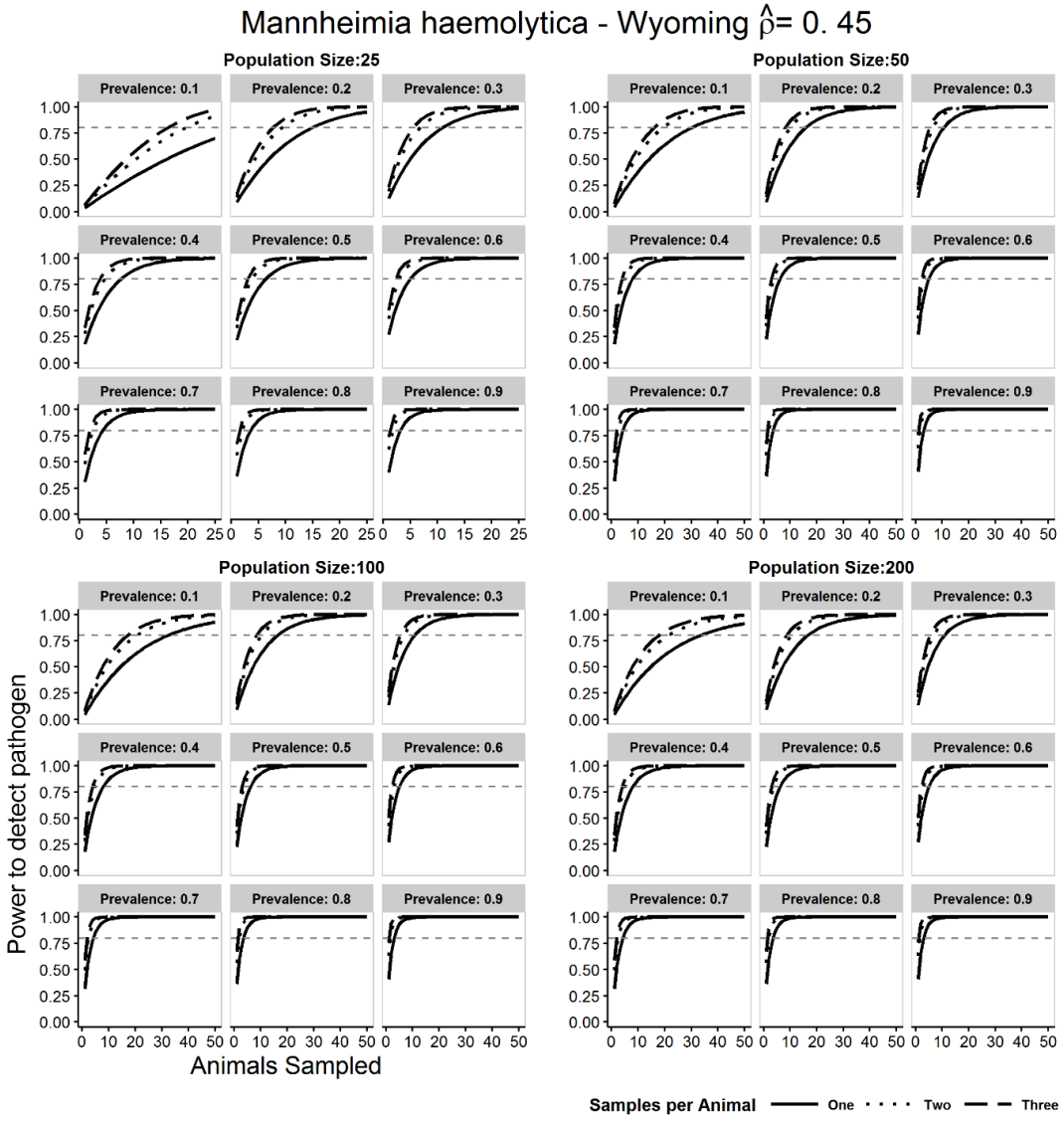

Bibersteinia trehalosi - Plated Culture* $\hat{\rho}$= 0. 1
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

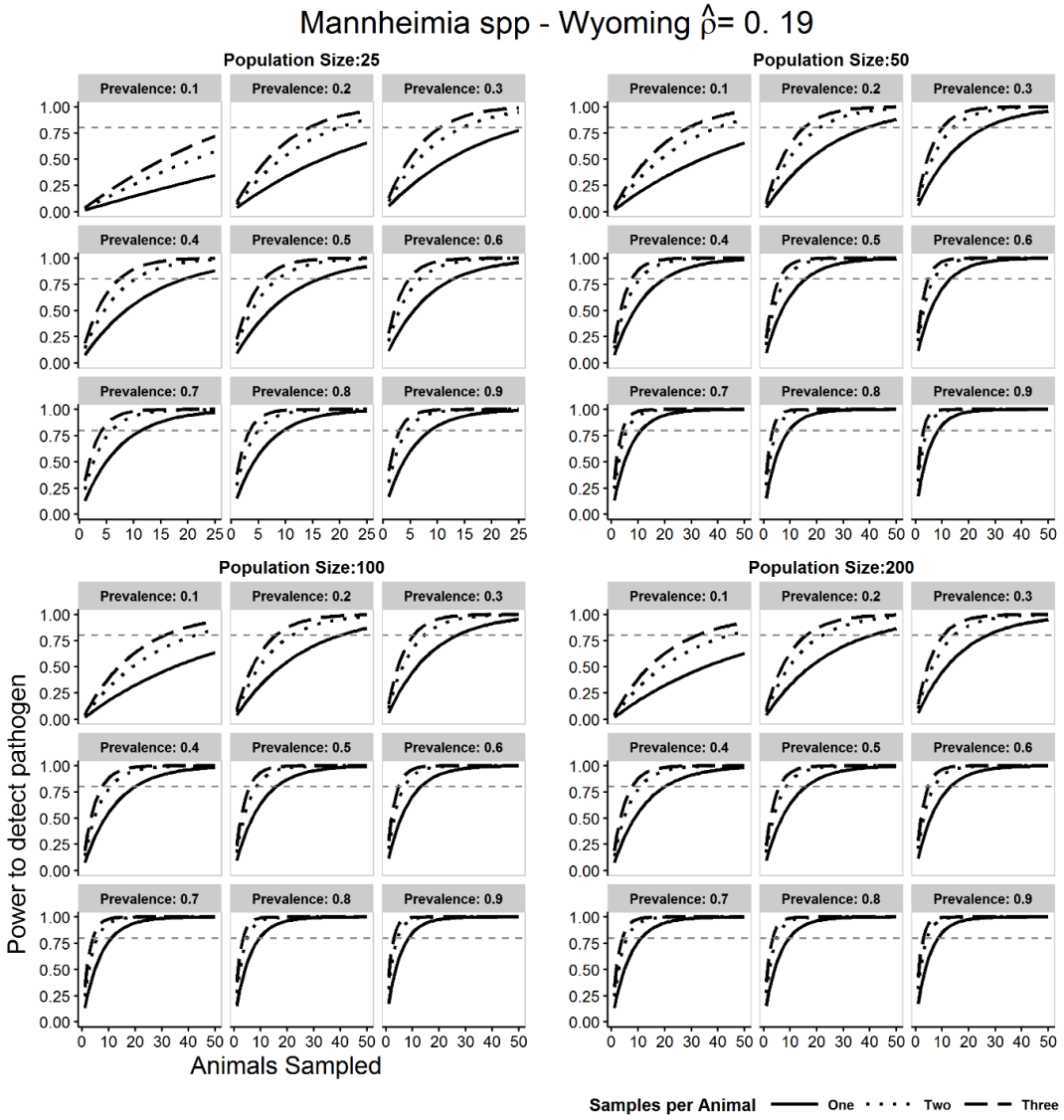
Plated PCR

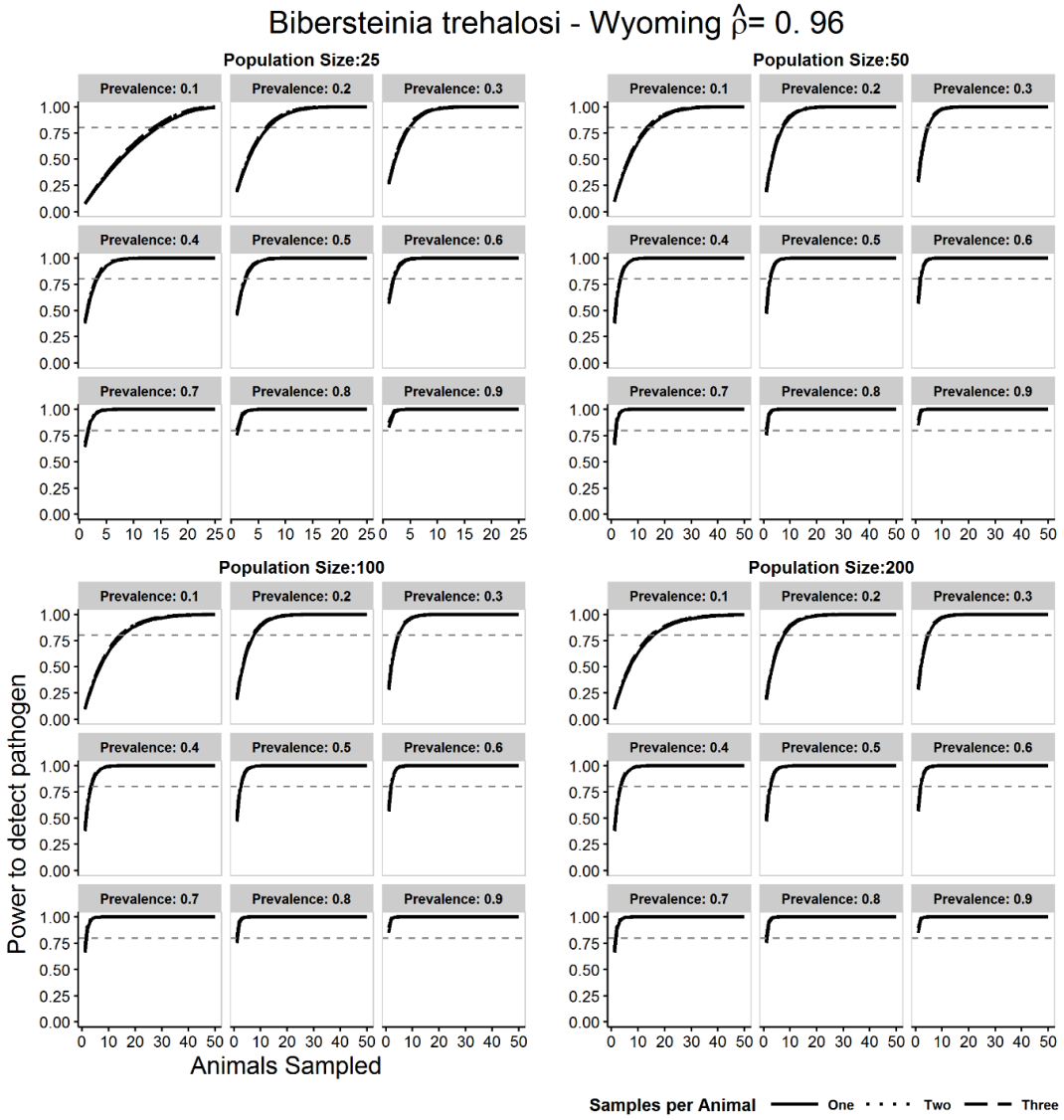

Mannheimia haemolytica - Plated PCR $\hat{\rho}$= 0. 26
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

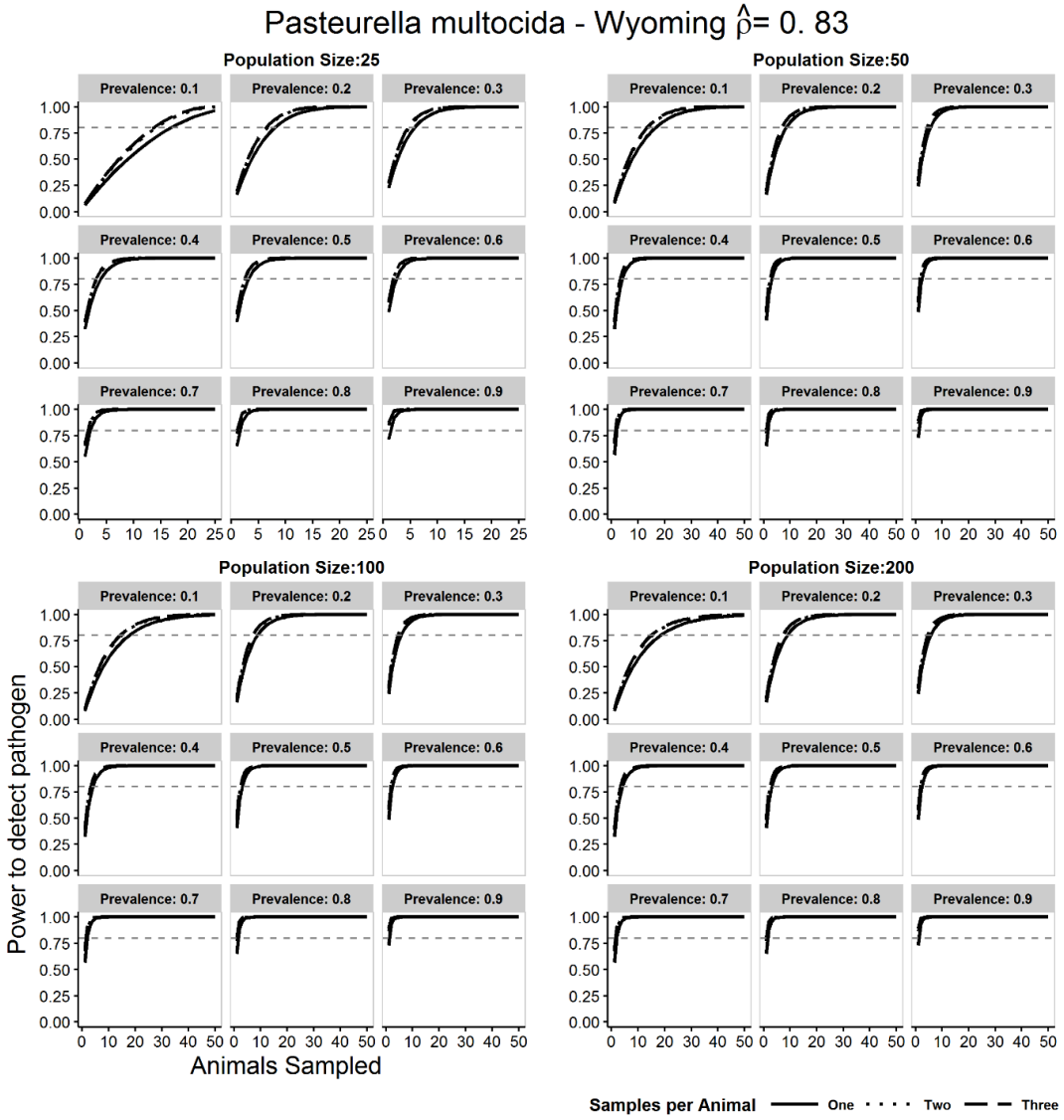

Mannheimia spp - Plated PCR $\hat{\rho}$= 0. 95
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

Wyoming


Mannheimia haemolytica - Wyoming $\hat{\rho}$= 0. 45
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three


Mannheimia spp - Wyoming $\hat{\rho}$= 0. 19
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three


Bibersteinia trehalosi - Wyoming $\hat{\rho}$= 0. 96
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three


Pasteurella multocida - Wyoming $\hat{\rho}$= 0. 83
Population Size:25
Population Size:50
Population Size:100
Population Size:200
Prevalence: 0.1
Prevalence: 0.2
Prevalence: 0.3
Prevalence: 0.4
Prevalence: 0.5
Prevalence: 0.6
Prevalence: 0.7
Prevalence: 0.8
Prevalence: 0.9
Power to detect pathogen
Animals Sampled
Samples per Animal
One
Two
Three

APPENDIX E

RESPIRATORY PATHOGEN SAMPLING INFORMATION

Table E1. Animals sampled by population, year, and set of diagnostic protocols that were used to detect *Pasteurellaceae* pathogens. The numbers under diagnostic protocols indicate the number of times the specified protocol was conducted per individual. Different rows within the same population and year represent "cohorts" of animals that were sampled for respiratory pathogens using the same suite of diagnostic protocols.

			Diagnostic Protocols Used per Animal					
Population	**Year**	**Animals Sampled**	Wyoming	Plated PCR	Plated Culture	Port-A-Cul	TSB	Tissue
Castle Reef	14.15	2	0	0	0	0	1	0
		5	0	1	0	0	1	0
		16	0	1	1	0	1	0
	15.16	1	0	0	2	0	2	0
		1	0	1	0	0	0	0
		6	0	1	2	0	2	0
	Total	**31**						
Dubois Badlands	13.14	5	1	0	0	0	0	0
	15.16	1	1	0	0	0	0	0
		4	1	0	0	0	1	0
	Total	**10**						
Fergus	14.15	30	0	0	0	0	1	0
		15	0	1	0	0	1	0
		15	0	1	1	0	1	0
	Total	**60**						
Franc's Peak	12.13	2	1	0	0	0	1	0
	13.14	12	1	0	0	0	0	0
	14.15	10	1	0	0	0	0	0
		3	1	0	1	0	1	0
	15.16	1	1	0	0	0	0	0
		1	1	0	0	0	1	0
	Total	**29**						
Highlands	15.16	16	0	0	0	0	2	0
	Total	**16**						
Hilgard	11.12	11	0	0	0	1	0	0
	13.14	29	0	0	0	0	1	0
	14.15	1	0	0	0	0	0	1
		1	0	0	0	0	1	0
		10	0	0	0	0	2	0
		20	0	1	0	0	2	0
		18	0	1	1	0	2	0
	15.16	3	0	1	0	1	2	0
		1	0	1	1	1	2	0

			Diagnostic Protocols Used per Animal					
Population	**Year**	**Animals Sampled**	Wyoming	Plated PCR	Plated Culture	Port-A-Cul	TSB	Tissue
		1	0	1	2	1	1	0
		29	0	1	2	1	2	0
	Total	**124**						
Jackson	12.13	15	1	0	0	0	0	0
		1	1	0	0	0	1	0
	13.14	12	1	0	0	0	0	0
	14.15	12	1	0	0	0	0	0
	15.16	11	1	0	0	0	0	0
		16	1	0	0	0	1	0
	Total	**67**						
Lost Creek	14.15	13	0	1	1	0	1	0
	15.16	1	0	0	2	0	2	0
		5	0	1	2	0	2	0
	Total	**19**						
Malta	15.16	19	0	0	0	0	2	0
	Total	**19**						
Paradise	14.15	2	0	0	0	0	1	0
		13	0	1	0	0	1	0
		15	0	1	1	0	1	0
	Total	**30**						
Petty Creek	15.16	1	0	1	2	1	0	0
		16	0	1	2	1	2	0
	Total	**17**						
Stillwater	14.15	16	0	1	1	0	1	0
	15.16	3	0	1	0	0	2	0
	Total	**19**						
Trout Peak	12.13	4	1	0	0	0	1	0
	13.14	9	1	0	0	0	0	0
	14.15	1	0	0	0	0	2	0
		2	1	0	0	0	0	0
	15.16	1	1	0	0	0	0	0
		7	1	0	0	0	1	0
	Total	**24**						
Wapiti Ridge	12.13	16	1	0	0	0	1	0
	13.14	13	1	0	0	0	0	0
	14.15	3	1	0	0	0	0	0
		7	1	0	1	0	1	0
	15.16	10	1	0	0	0	0	0

Population	Year	Animals Sampled	Diagnostic Protocols Used per Animal					
			Wyoming	Plated PCR	Plated Culture	Port-A-Cul	TSB	Tissue
		10	1	0	0	0	1	0
	Total	**59**						
Whiskey Mountain	13.14	3	1	0	0	0	0	0
	14.15	20	1	0	0	0	0	0
	15.16	16	1	0	0	0	0	0
		12	1	0	0	0	1	0
	Total	**51**						
Upper Yellowstone Pre-2013	11.12	6	0	0	0	1	0	0
	12.13	11	0	0	0	0	1	0
	Total	**17**						
Upper Yellowstone Post-2013	12.13	2	0	0	0	1	0	0
	13.14	5	0	0	0	0	1	0
	14.15	15	0	0	0	0	0	1
	Total	**22**						
Clark's Fork	12.13	1	0	0	0	0	1	0
		8	1	0	0	0	1	0
	13.14	3	1	0	0	0	0	0
	14.15	8	1	0	0	0	0	0
	Total	**20**						

Table E2. Animals sampled by population, year, and set of diagnostic protocols that were used to detect *Mycoplasma ovipneumoniae*. The numbers under diagnostic protocols indicate the number of times the specified protocol was conducted per individual. Different rows within the same population and year represent "cohorts" of animals that were sampled for respiratory pathogens using the same suite of diagnostic protocols

			Diagnostic Protocols			
Population	**Year**	**Animals Sampled**	Wyoming	TSB	qPCR	Tissue
Castle Reef	14.15	8	0	1	0	0
		15	0	1	1	0
	15.16	7	0	2	0	0
	Total	**30**				
Dubois Badlands	13.14	5	1	0	0	0
	15.16	5	1	1	0	0
	Total	**10**				
Fergus	14.15	30	0	1	0	0
		29	0	1	1	0
	Total	**59**				
Franc's Peak	12.13	2	1	1	0	0
	13.14	12	1	0	0	0
	14.15	13	1	0	0	0
	15.16	2	1	1	0	0
	Total	**29**				
Highlands	15.16	16	0	2	0	0
	Total	**16**				
Hilgard	11.12	11	0	1	0	0
	13.14	29	0	1	0	0
		1	0	0	0	1
		50	0	1	0	0
	15.16	35	0	2	0	0
	Total	**126**				
Jackson	12.13	1	0	1	0	0
		13	1	0	0	0
	13.14	13	1	0	0	0
	14.15	12	1	0	0	0
		7	1	0	0	0
	15.16	16	1	1	0	0
	Total	**62**				
Lost Creek	14.15	6	0	1	0	0
		7	0	1	1	0
	15.16	6	0	2	0	0

Population	Year	Animals Sampled	Diagnostic Protocols			
			Wyoming	TSB	qPCR	Tissue
	Total	**19**				
Malta	15.16	19	0	2	0	0
	Total	**19**				
Paradise	14.15	30	0	1	1	0
	Total	**30**				
Petty Creek	15.16	16	0	2	0	0
	Total	**16**				
Stillwater	14.15	9	0	1	0	0
		7	0	1	1	0
	15.16	3	0	2	0	0
	Total	**19**				
Trout Peak	12.13	4	1	1	0	0
	13.14	9	1	0	0	0
	14.15	2	0	1	0	0
		2	1	0	0	0
	15.16	8	1	1	0	0
	Total	**25**				
Wapiti Ridge	12.13	16	1	1	0	0
	13.14	13	1	0	0	0
	14.15	10	1	0	0	0
	15.16	3	1	0	0	0
		14	1	1	0	0
	Total	**56**				
Whiskey Mountain	13.14	3	1	0	0	0
	14.15	20	1	0	0	0
	15.16	11	1	0	0	0
		14	1	1	0	0
	Total	**48**				
Upper Yellowstone Pre-2013	11.12	6	0	1	0	0
	12.13	11	0	1	0	0
	Total	**17**				
Upper Yellowstone Post-2013	12.13	2	0	1	0	0
	13.14	5	0	1	0	0
	14.15	15	0	0	0	1
	Total	**22**				
Clark's Fork	12.13	6	0	1	0	0
		2	1	0	0	0

Population	Year	Animals Sampled	Diagnostic Protocols			
			Wyoming	TSB	qPCR	Tissue
		1	1	1	0	0
	13.14	3	1	0	0	0
	14.15	8	1	0	0	0
	Total	**20**				

Assessing Detection Power When Protocols
Without Detection Probability Estimates Were Used

Detection probability for one pathogen-protocol combination (*B. trehalosi*-Plated PCR) could not be reliably estimated because the pathogen was only detected in two animals where the protocol was used. Both detections were from the Plated PCR protocol suggesting detection probability is higher than for the other protocols used on the same set of animals. When estimating probability that *B. trehalosi* was present in the populations where it was not detected and the Plated PCR protocol was employed, the detection probability parameters for the TSB protocol (the protocol with the highest estimable detection probability that was also used on the same set of individuals) were used for the Plated PCR protocol, and when reporting the detection power for *B. trehalosi* it was denoted that the true power was more than reported. Detection probability for several pathogen-protocol combinations could not be estimated because the protocol never detected the pathogen (*Mannheimia* spp.-Port-A-Cul, *B. trehalosi*-Port-A-Cul, *P. multocida*-Plated Culture), suggesting lower detection probability than for protocols that did detect these pathogens. For populations where these protocols were applied and the specified pathogen was not detected, the detection power for these pathogens was estimated assuming detection probability for these pathogen-protocol combinations was

zero, but it was denoted that the true detection power was higher than estimated, since detection probability for these protocols was likely greater than zero. Where *M. ovipneumoniae* was not detected in a population where serology testing was also used to assess evidence for presence of the pathogen, it was denoted that true detection power was higher than estimated, since sensitivity of the serology test is unknown. Detection probabilities of any diagnostic test (e.g., culture or PCR) applied to lung tissues are unknown and this was reflected by using a uniform beta distribution ($\alpha=1$, $\beta=1$) to define detection probability when tissue samples were used to assess pathogen presence. This is only applicable to the Upper Yellowstone Complex in 2014/2015, where *B. trehalosi* and *Mannheimia* spp. were not detected.

Table E3. Detection probability parameters for *Pasteurellaceae* and *Mycoplasma ovipneumoniae* diagnostic protocols (adopted from Butler *et al. in press*).

Pathogen	Protocol	Distribution Parameters	
		Logit-Normal (μ,σ^2)	*Beta* (α, β)
Mannheimia haemolytica			
	Plated PCR	-1.07, 0.46	6.63, 18.36
	Plated Culture	-0.83, 0.44	8, 17.65
	Port-A-Cul	-2.18, 1.07	1.22, 7.34
	TSB	-1.02, 0.32	14.01, 37.82
	Wyoming PCR	-0.19, 0.49	8.08, 9.71
	Tissue[1]	--	1,1
Mannheimia spp.			
	Plated PCR	3.01, 1.02	16.11, 1.22
	Plated Culture	-2.99, 0.46	5.13, 93.05
	Port-A-Cul[2]	--	0,0
	TSB	-2, 0.2	27.96, 202.63
	Wyoming	-1.46, 0.28	16.66, 69.99
	Tissue[1]	--	1,1
Bibersteinia trehalosi			
	Plated PCR	---	23.28, 41.64
	Plated Culture	-2.15, 0.75	2.26, 15.79
	Port-A-Cul[2]	--	0,0
	TSB	-0.59, 0.26	23.28, 41.64
	Wyoming	3.29, 1.02	20.42, 1.2
	Tissue[1]	--	1,1
Pasteurella multocida			
	Plated Culture[2]	---	0,0
	Port-A-Cul	-0.25, 1.29	1.48, 1.77
	TSB	-1.9, 0.26	16.84, 110.23
	Wyoming	1.58, 0.63	14.48, 3.36
	Tissue[1]	--	1,1
Mycoplasma ovipneumoniae			
	qPCR	0.59, 0.69	6.28, 3.69
	TSB	0.98, 0.25	60.18, 22.91
	Wyoming PCR	1.85, 0.53	25, 4.36
	Tissue[1]	--	1,1

[1.] There were no estimates of detection probability for lung tissue samples and a uniform beta distribution was used to reflect the lack of information.

[2.] Detection probability could not be directly estimated because these protocols never detected the respective pathogens. Detection probability in these instances was set at zero.

APPENDIX F

DEMOGRAPHIC DATA SUMMARIES

Table F1. Summary of demographic data for 17 bighorn sheep study populations in Montana and Wyoming that were investigated as part of this study

Pop. Name	Pop Est.	Meets Population Objective[1]	10 Year Trend	5 Yr. Mean (SD) Lamb:ewe Ratio	5 Yr. Mean (SD) Ewe Harvest	5 Yr. Mean (SD) Ram Harvest	5 Yr. Mean (SD) Translocations[2]
Perma-Paradise[3]	325	Yes	Stable[1]	0.34 (0.05)	3 (1.63)	13.75 (1.26)	4.4 (8.8)
Petty Creek	160	Yes	Stable	0.43 (0.06)	5.8 (4.09)	4 (3)	0 (0)
Lost Creek	100	No	Decline	0.1 (0.07)	0 (0)	1.4 (1.95)	0 (0)
Highlands	75	No	--	--	0 (0)	0.4 (0.49)	1.8 (4.4)
Castle Reef	150	No	Decline	0.09 (0.04)	1 (1.73)	3.6 (0.89)	0 (0)
Fergus	545	Yes	Increase	0.29 (0.02)	16 (2.83)	16 (0)	-15 (21.21)
Middle Missouri Breaks	400	Yes	Increase	0.47 (0.09)	12.25 (10.24)	8 (1.41)	0 (0)
Hilgard	280	Yes	Increase	0.4 (0.23)	5 (4.58)	3.2 (1.3)	-14.8 (20.45)
Upper Yellowstone	320	Yes	Increase	0.34 (0.06)	0 (0)	3.8 (2.17)	0 (0)
Stillwater	75	Yes	Increase	0.43 (0.12)	0 (0)	1.2 (0.84)	0 (0)
Clark's Fork	600	Yes	Increase	0.33 (0.14)	0 (0)	17.25 (1.5)	0 (0)
Trout Peak	700	Yes	Stable	0.27 (0.03)	0 (0)	18 (2.65)	0 (0)
Wapiti Ridge	850	Yes	Decline	0.26 (0.09)	0 (0)	35.2 (3.56)	0 (0)
Franc's Peak	840[4]	No[4]	Decline	0.26 (0.07)	0 (0)	55 (20.21)	0 (0)
Dubois Badlands			Decline	0.19 (0.12)	0 (0)	1.5 (1.29)	0 (0)
Whiskey Basin	1000	No	Increase	0.30 (0.08)	0 (0)	15 (1.22)	0 (0)
Jackson	425	No	Stable	0.31 (0.08)	0 (0)	8.6 (1.82)	0 (0)

1. Meeting population objective defined as ≥ 80% of listed population objective for Wyoming populations and ≥ 90% of listed population objective for Montana populations pursuant to state and population-specific management goals.
2. Negative sign indicates translocations out of population and a positive sign indicates translocations into population.
3. Population was purposefully reduced through harvest and translocation. Trend considered stable.
4. A single population estimate and objective is established for Franc's Peak and Dubois Badlands by Wyoming Game and Fish Department.

Piecewise Regression to Identify Break-Points in Recruitment Rates

A limitation to linking numerous years of recruitment data to respiratory pathogen data only collected during a subset of those years is that the potential exists for pathogens to have been introduced to or gone extinct from the populations within the recruitment data time-series. Therefore, the average recruitment rate of a population could be composed of recruitment data obtained from years where certain respiratory pathogens were present and years where they were not, compromising the ability to accurately characterize recruitment with respect to pathogen presence. It was reasoned that the introduction or extinction of any respiratory pathogen that affects lamb survival should result in a pronounced change in recruitment rates of the population after the change, as lamb survival is the vital rate most likely to be affected by respiratory disease [52]. To identify years where an influential respiratory pathogen may have been introduced to or gone extinct from study populations, a piecewise regression on lamb:ewe ratios was conducted for each study population using data collected from 2006-2016. Lamb:ewe ratios from each population were iteratively assigned to one of two groups depending on whether they were observed before or after each year the dataset [74], and the AIC_C of the following Poisson regression model was obtained for each year-based grouping, *i*:

$$\mu = \beta_0 + \beta_1 \cdot (Year > i),$$

where μ is the mean $log\left(\frac{lambs}{ewes}\right)$. A Poisson regression model was chosen to reflect that the index of recruitment was obtained from count data. The iterative splitting of

observations into groups was halted prior to the most recent year of recruitment data to prevent splitting based on a single year's recruitment rate. The AIC_C scores of these models, as well as that of a single mean model, were ranked and AIC_C scores used to determine if the data were best modeled with a single-mean regression line or two-mean regression lines breaking at a specific year within the time series for each population. Although this procedure doesn't exclude the possibility that introduction or extinction of respiratory pathogens didn't occur within the resulting datasets, the unobserved occurrence of such events without causing a shift in recruitment rates would suggest whatever changes in pathogen community that occurred were not important.

Table F2. Break-years and mean lamb:ewe ratios before and after the break year for each population as estimated by piecewise regression. Lamb:ewe ratios for each population were iteratively split into "before" and "after" groups for every year in the dataset and a Poisson regression model to estimate average lamb:ewe ratios for the "before" and "after" groups was run for each year-based grouping. The break-year was chosen as the year-based grouping with the lowest AIC_C score. Break-years were estimated based on before and after recruitment-rates to detect potential years where respiratory pathogens may have been introduced or gone extinct from study populations. Years of documented all-age respiratory disease epizootics since 2006 are also shown for reference.

Population	**Documented All-age Epizootics**	**Break year**	**Mean lamb:ewe ratio (95% confidence interval)**	
			Before break year	*After break year*
Castle Reef	2010	2010	0.28 (0.25-0.33)	0.08 (0.06-0.11)
Clark's Fork	--	2014	0.33 (0.28-0.39)	0.22 (0.15-0.31)
Dubois Badlands	--	2010	0.3 (0.25-0.35)	0.16 (0.11-0.22)
Fergus	--	None	0.29 (0.22-0.38)	--
Franc's Peak	2011-2013	2011	0.32 (0.29-0.34)	0.24 (0.21-0.27)
Hilgard	--	2012	0.24 (0.2-0.3)	0.45 (0.39-0.52)
Jackson	2012	2011	0.42 (0.38-0.46)	0.32 (0.28-0.36)
Lost Creek	2010	2010	0.42 (0.37-0.49)	0.11 (0.07-0.15)
Middle Missouri Breaks	--	None	0.46 (0.41-0.51)	--
Paradise	--	None	0.35 (0.32-0.38)	--
Petty Creek	--	2010	0.34 (0.27-0.42)	0.44 (0.38-0.52)
Stillwater	--	2013	0.34 (0.27-0.42)	0.51 (0.38-0.67)
Trout Peak	--	None	0.28 (0.26-0.31)	--
Wapiti Ridge		2010	0.3 (0.28-0.33)	0.23 (0.21-0.26)
Whiskey Basin	--	2012	0.25 (0.23-0.27)	0.31 (0.28-0.35)
Upper Yellowstone	2012,2014	2010	0.28 (0.25-0.33)	0.35 (0.32-0.39)

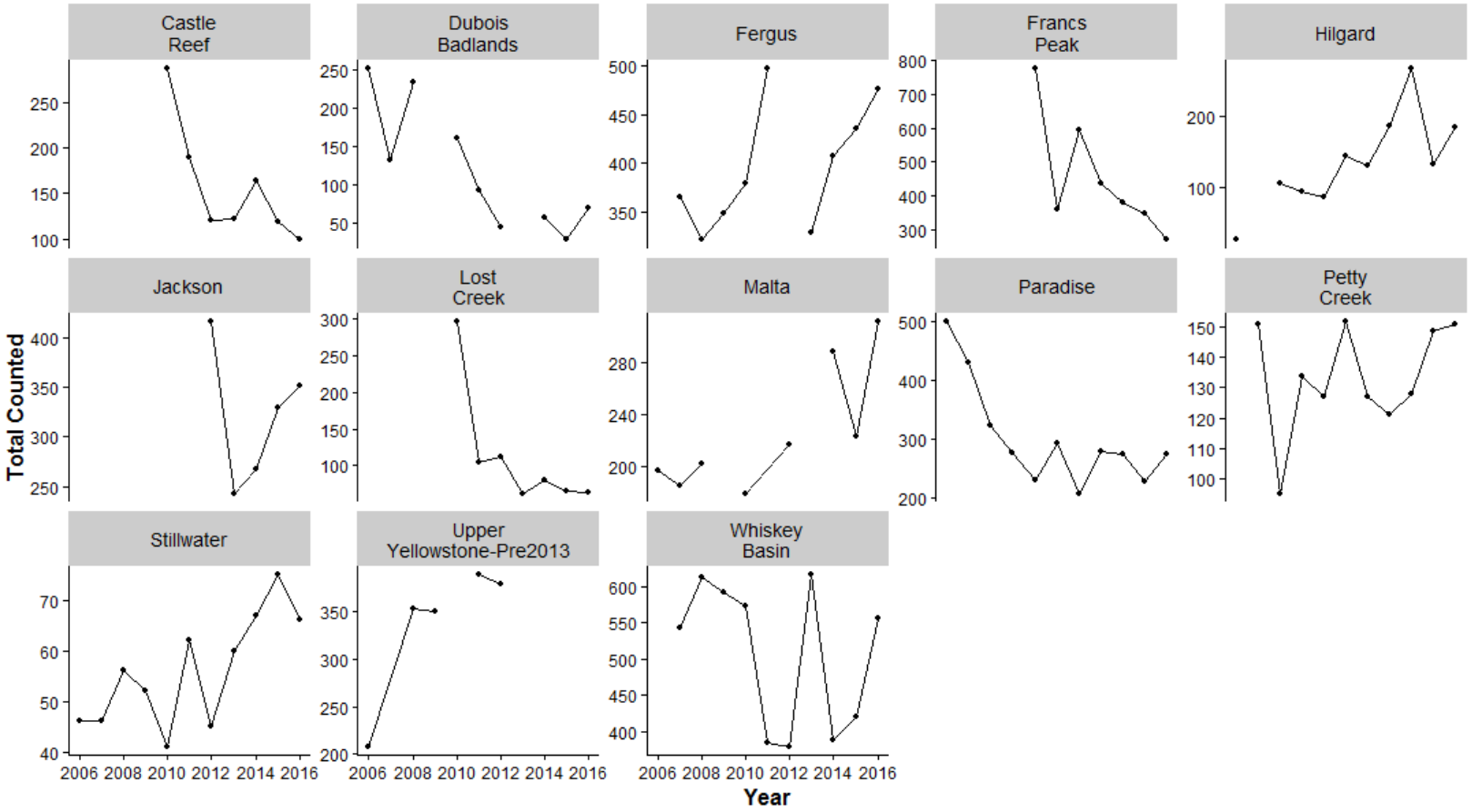


Figure F1. Raw annual population counts of 13 bighorn sheep populations in Montana and Wyoming that were investigated as part of this study. The y-axes show the total number of animals counted and the x-axes show the years the counts were conducted. The y-axes change across panels to illustrate population trends.

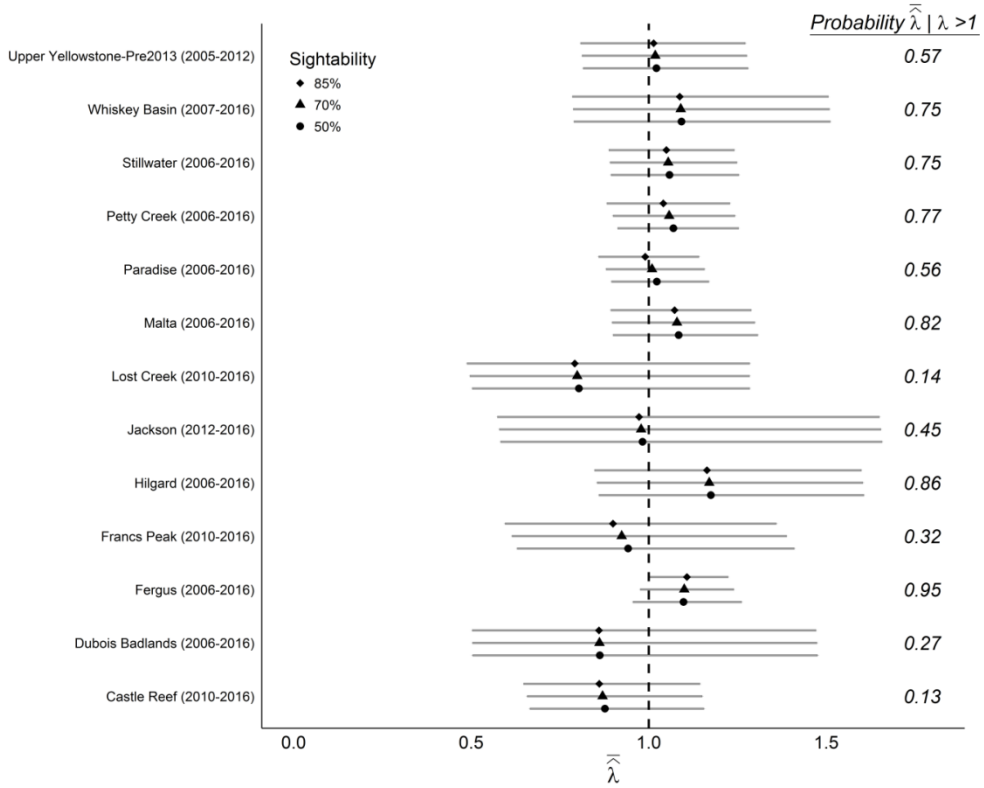


Figure F2. Geometric mean lambda and associated 95% confidence intervals for 13 bighorn sheep populations, after accounting for harvest and translocation. The three points and intervals for each study population represent estimates assuming 50%, 70%, or 85% sightability in population surveys. The confidence that population growth was positive ($\bar{\lambda}$> 1) for each study population, assuming 70% sightability, is shown on the right side of the plot.