PLOS ONE

RESEARCH ARTICLE

Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia

Thomas E. Besser[1]*, E. Frances Cassirer[2], Kathleen A. Potter[1], William J. Foreyt[1]

1 Department of Veterinary Microbiology and Pathology, Washington State University College of Veterinary Medicine, Pullman, WA, United States of America, **2** Idaho Department of Fish and Game, Lewiston, ID, United States of America

* tbesser@vetmed.wsu.edu

OPEN ACCESS

Citation: Besser TE, Cassirer EF, Potter KA, Foreyt WJ (2017) Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia. PLoS ONE 12(6): e0178707. https://doi.org/10.1371/journal.pone.0178707

Editor: Marco Festa-Bianchet, Université de Sherbrooke, CANADA

Received: February 26, 2017

Accepted: May 17, 2017

Published: June 7, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Wild Sheep Foundation / Foundation for North American Wild Sheep Grant-in-Aid funding to T. Besser (https://www.wildsheepfoundation.org/). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Background

Bronchopneumonia is a population limiting disease of bighorn sheep (*Ovis canadensis*) that has been associated with contact with domestic Caprinae. The disease is polymicrobial but is initiated by *Mycoplasma ovipneumoniae*, which is commonly carried by both domestic sheep (*O. aries*) and goats (*Capra aegagrus hircus*). However, while previous bighorn sheep comingling studies with domestic sheep have resulted in nearly 100% pneumonia mortality, only sporadic occurrence of fatal pneumonia was reported from previous comingling studies with domestic goats. Here, we evaluated the ability of domestic goats of defined *M. ovipneumoniae* carriage status to induce pneumonia in comingled bighorn sheep.

Methodology/Principal findings

In experiment 1, three bighorn sheep naïve to *M. ovipneumoniae* developed non-fatal respiratory disease (coughing, nasal discharge) following comingling with three naturally *M. ovipneumoniae*-colonized domestic goats. Gross and histological lesions of pneumonia, limited to small areas on the ventral and lateral edges of the anterior and middle lung lobes, were observed at necropsies conducted at the end of the experiment. A control group of three bighorn sheep from the same source housed in isolation during experiment 1 remained free of observed respiratory disease. In experiment 2, three bighorn sheep remained free of observed respiratory disease while comingled with three *M. ovipneumoniae*-free domestic goats. In experiment 3, introduction of a domestic goat-origin strain of *M. ovipneumoniae* to the same comingled goats and bighorn sheep used in experiment 2 resulted in clinical signs of respiratory disease (coughing, nasal discharge) in both host species. At the end of experiment 3, gross and histological evidence of pneumonia similar to that observed in experiment 1 bighorn sheep was observed in both affected bighorn sheep and domestic goats.

Conclusions/Significance

M. ovipneumoniae strains carried by domestic goats were transmitted to comingled bighorn sheep, triggering development of pneumonia. However, the severity of the disease was

markedly milder than that seen in similar experiments with domestic sheep strains of the bacterium.

Introduction

Historical and contemporary field observations suggest that contacts with domestic sheep are followed by pneumonia outbreaks in previously healthy bighorn sheep populations [1±3]. Supporting these observations, >95% of 90 bighorn sheep in eleven studies involving contact with domestic sheep suffered fatal pneumonia within 100 days, as reviewed in [4]. However, the potential role of domestic goats in transmitting pneumonia pathogens to bighorn sheep is less clear, with relatively fewer observed bighorn sheep pneumonia outbreaks following domestic goat contacts [5±7] and with relatively little fatal bighorn sheep pneumonia observed in two experimental comingling studies with domestic goats (22% of 9 bighorn sheep) [5, 8]. Domestic goats may transmit respiratory pathogens when they contact bighorn sheep on or near bighorn sheep home ranges as stray animals from farmsteads, or when used for weed control, or when commmercial operations are grazed on public lands in or near bighorn sheep home ranges, or when used as pack animals supporting back-country recreation. In addition, bighorn sheep may encounter domestic goats on private lands within or adjacent to bighorn ranges. Therefore, it is important to understand the risk that contact with domestic goats may pose for bighorn sheep health.

Mycoplasma ovipneumoniae, proposed as the primary pathogen that triggers bighorn sheep pneumonia outbreaks [9, 10] followed by persistently impaired recruitment resulting from recurrent lamb pneumonia [11, 12], has a host range limited to Caprinae [13]. Distinct host-specific clades of *M. ovipneumoniae* have recently been reported in domestic sheep and goats [7, 14]. In a Washington state survey of goat farms adjacent to bighorn sheep habitat, this pathogen was carried asymptomatically by animals on 7 of 16 goat farms, and by 58% of individual goats on positive farms [15].

Like bighorn sheep pneumonia, respiratory disease of domestic sheep and goats is polymicrobial, frequently including *M. ovipneumoniae* (and perhaps also *Mycoplasma arginini*), *Mannheimia haemolytica*, *Pasteurella multocida*, and other pathogens. Chronic and persistent coughing is the main clinical sign observed associated with *M. ovipneumoniae* infection of domestic lambs, which is often described as summer pneumonia in Australia and New Zealand, or as coughing syndrome in the United States [13, 16]. Clinical severity ranges from mild illness associated with reduced growth rates to fatal polymicrobial pneumonia [13, 17]. *M. ovipneumoniae* has also been associated with outbreaks of severe respiratory disease in goats [13, 18, 19].

The *M. ovipneumoniae* carriage status of the animals used in previously published experimental domestic goat-bighorn sheep comingling studies was not defined [5, 8] and so it is possible that the low death rate reported in those studies was due to the absence of *M. ovipneumoniae* in the comingled goats. Here we tested the hypothesis that exposure to domestic goats carrying *M. ovipneumoniae* results in epizootic pneumonia in bighorn sheep by conducting a series of experimental comingling studies with domestic goats of defined *M. ovipneumoniae* carriage status.

Materials and methods

Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and in conformance with

United States Department of Agriculture animal research guidelines, under protocols #03793 and #04482 approved by the Washington State University Institutional Animal Care and Use Committee. As described in those protocols, euthanasia was to be performed by intravenous injection of sodium pentobarbital for animals observed to be in severe distress associated with pneumonia during the study and prior to any necropsy examination for surviving animals at the end of each experiment.

Experimental animals

Six healthy, *M. ovipneumoniae*-unexposed, pregnant adult bighorn ewes were provided by the Washington Department of Fish and Wildlife on January 25, 2014 from the Clemans Mountain population for these studies. This population had previously been confirmed to be uninfected and unexposed to *M. ovipneumoniae* as determined by repeated testing with both competitive enzyme-linked immunosorbent assay (cELISA) serology and nasal swab polymerase chain reaction (PCR) testing [20]. Blood and nasal swab samples collected from the six experimental animals on the day of capture were also negative by *M. ovipneumoniae* cELISA and PCR tests.The ewes were randomly allocated to two experimental groups of three animals each. Sampling or moving the bighorn sheep was conducted under anesthesia (butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride, reversed with atipamezole and naltrexone, BAM kit, Wildlife Pharmaceuticals, Inc. Windsor CO). Six domestic goats were purchased from three local private owners for use in the experiments. Domestic goats used in experiment 1 (group 1) included a 5+ year Saanen wether previously used as a pack animal, a 1 year old mixed breed wether intended for slaughter, and a 1 year old Boer wether from a herd used for weed and brush control. All were confirmed nasal carriers of *M. ovipneumoniae* based on PCR testing of nasal swab samples on at least two occasions prior to the study. Domestic goats used in experiments 2 and 3 (group 2) included one yearling Boer female and two yearling LaMancha wethers, all negative for *M. ovipneumoniae* by nasal swab PCR when purchased. Because they originated in a *M. ovipneumoniae*-positive herd, group 2 domestic goats were placed in isolation from other small ruminants and were serially re-tested for *M. ovipneumoniae* nasal carriage (5 times over a 4 week period) to confirm their negative status prior to the start of experiment 2.

Experimental design

Three experiments were conducted in which domestic goats (DG) of known *M. ovipneumoniae* status were comingled with susceptible bighorn sheep (BHS). In each experiment, comingled animals shared feed and water sources (pelleted hay supplemented with a corn/oats/barley mixture, free choice trace mineral salt, and a single frost-protected water source in each pen). Single roofed shelters were available in each pen, and plywood sheets covered the pen fences at the corners to provide windbreaks. All animals were observed daily.

Experiment 1. BHS group 1 (BHS29, BHS32, and BHS33; *M. ovipneumoniae*-negative) was comingled in a 372 m^2 pen (pen 1) with group 1 DG (DG1, DG2, and DG3; *M. ovipneumoniae*-positive) beginning February 18, 2014. During experiment 1, BHS group 2 (BHS27, BHS28, and BHS31; *M. ovipneumoniae*-negative) were housed in a separate 232 m^2 pen (pen 2) isolated >30 m from any other domestic or wild sheep or goats. All animals were observed closely for signs of respiratory disease for a 100 day period. On May 30, 2014, BHS group 1 animals were euthanized and subjected to complete necropsy examinations. DG group 1, having shown no clinical signs of respiratory disease during the experiment, were donated to the Washington State University veterinary teaching program.

Experiment 2. After group 1 animals were removed at the end of experiment 1, pen 1 was left empty for 40 days before BHS group 2 (*M. ovipneumoniae*-negative) was added on July 7,

2014. BHS group 2 composition was modified by the loss of BHS27 and by the addition of BHS28L (a lamb born to BHS28 on April 18, 2014 during the first experiment. On September 10, 2014, 65 days after BHS group 2 were placed in pen 1, DG group 2 (DG4, DG5, and DG6; *M. ovipneumoniae*-negative) was comingled to begin experiment 2, in which the comingled animals were again closely observed for signs of respiratory disease for a 100 day period.

Experiment 3. In experiment 3, a strain of *M. ovipneumoniae* obtained by nasal wash from a domestic goat (originating on a different farm than the source farms of DG groups 1 and 2) was introduced into the comingled BHS and DG from experiment 2, as follows: DG4 and DG5 were removed to an isolation pen where they were each inoculated with *M. ovipneumoniae* by instillation of 10 ml of ceftiofur-treated nasal wash fluids into conjunctivae and nasal passages. After confirmation of colonization by positive nasal swab PCR tests on days 3 and 7 following inoculation, they were re-commingled in pen 1 with *M. ovipneumoniae*-negative DG6 and BHS group 2 on February 16, 2015. The commingled animals were observed closely for signs of respiratory disease for a 100 day period. At the end of experiment 3, both BHS goup 2 and DG group 2 were euthanized and subjected to complete necropsy examinations, as all had demonstrated signs of respiratory disease during the experiment.

Biosecurity

The study pens used for these experiments had not housed any BHS, DG or domestic sheep for >12 months prior to initiation of these experiments. Routine biosecurity measures during the experiments included broad physical separation (>1 km) and entirely separate animal care staff for pens 1 and 2. Animal care staff and researchers utilized personal clean protective equipment (coveralls, boots, and gloves) when entering study pens. Research staff occasionally entered both pens 1 and 2 on the same day, but if so always entered the *M. ovipneumoniae*-free pen 2 first, and changed personal protective equipment before entering pen 1.

Clinical scores

Clinical signs of respiratory disease were scored appproximately 3 times per week using a point system: anorexia (1 point), nasal discharge (1 point), cough (2 points), nose licking (1 point), head shaking (1 point), ear paresis (1 point) and weakness/incoordination (1 point), as we have done previously [21].

Microbiological testing

Routine microbiological testing performed by the Washington Animal Diagnostic Laboratory (WADDL, accredited by the American Association of Veterinary Laboratory Diagnosticians), included detection of *M. ovipneumoniae*-specific antibodies in serum samples using competitive enzyme-linked immunosorbent assays (cELISA) [9, 22], detection of *M. ovipneumoniae*-specific DNA sequences by polymerase chain reaction (PCR) testing of nasal or bronchial swab eluates, culture-enriched swab samples (mycoplasma broth, 72 hrs, 35 C), or lung tissue samples [23, 24], detection of Pasteurellaceae in pharyngeal swab samples by conventional aerobic bacteriologic cultures and MALDI-TOF (Biotyper, Bruker, Woodlands TX) [25], and detection of neutralizing antibodies in serum samples indicating exposure to parainfluenza-3 and respiratory syncytial viruses by virus neutralization assays. PCR tests specific for detection of *M. haemolytica*, *B. trehalosi*, and *P. multocida*, and *lktA* (the gene encoding the principal virulence factor of *M. haemolytica* and *B. trehalosi*) were applied to DNA extracted from pneumonic lung tissues using previously described methods [26]. The 16S±23S ribosomal operon intergenic spacer (IGS) regions of *M. ovipneumoniae* recovered from animals in these studies

were PCR amplified and their DNA sequences determined in order to track transmission of specific *M. ovipneumoniae* strains (GenBank accession numbers pending) [7, 27].

Results

Experiment 1

All animals appeared clinically normal at the start of experiment 1. All BHS were negative for carriage (PCR testing of nasal swabs) and exposure to *M. ovipneumoniae* (cELISA testing of serum samples) (Table 1). DG were confirmed as carriers of *M. ovipneumoniae* based on detection of the bacterium by PCR on repeated nasal swab samples. Diverse pharyngeal Pasteurellaceae were detected in both BHS and DG during experiment 1 (Table 1). Group 1 BHS (BHS29, BHS32, and BHS33) and group 1 DG (DG1, DG2, and DG3) were commingled in pen 1 to begin the experiment, while group 2 BHS (BHS27, BHS28, and BHS31) were maintained in isolation in pen 2. Beginning 2±3 weeks after commingling, all group 1 BHS began showing signs of respiratory disease, including increased nasal discharge and coughing (Fig 1, S1 Table). Clinical signs of middle ear involvement (head shaking, ear droop) were rarely seen. After 70 days of illness, group 1 BHS exhibited decreasing clinical signs of respiratory tract disease, and group 1 DG remained clinically normal throughout the experiment. Group 1 BHS were euthanized and necropsied after 100 days of commingling. Uncommingled group 2 BHS remained healthy in pen 2 throughout experiment 1. At the end of experiment 1, the *M. ovipneumoniae*-negative status of group 2 BHS was re-confirmed by PCR and cELISA serology. BHS27 subsequently died from aspiration pneumonia, the onset of which followed the anesthesia conducted to permit sampling after experiment 1; at necropsy, BHS27 was also PCR- and ELISA-negative for *M. ovipneumoniae*.

All BHS ewes lambed during experiment 1. All lambs born to group 1 (goat-commingled, *M. ovipneumoniae*-positive) BHS died at less than 7 days of age, with signs of ocular, systemic and/or gastrointestinal tract disease (BHS29L, BHS32L, and BHS33L; Table 2). At necropsy,

Table 1. Microbiological status and pathologic lesions of animals in experiment 1.

Animal	Pen	Stage	Gross necropsy[1]	*M. ovipneumoniae*[2]		*Pasteurellaceae (lktA)*[3]
				PCR	cELISA (%I)	
BHS29	1	Pre	-	NotDet	NotDet (-12%)	P: Btre, Mhae+
		Post	LC (15% r; 5% l), CSE	Det: B, CS	Det (75%)	L: Pmul
BHS32	1	Pre	-	NotDet	NotDet (-13%)	P: Btre+
		Post	LC (5% l), CSE	Det: N, L	Det (60%)	L: NotDet
BHS33	1	Pre	-	NotDet	NotDet (3%)	P: Mhae+
		Post	LC (1% r) PA ® CSE	Det: B, N	Det (82%)	L: Pspp
DG1	1	Both	-	Det: N	-	P: Btre, Mhae+
DG2	1	Both	-	Det: N	-	P: Mhae+
DG3	1	Both	-	Det: N	-	P: Btre, Mhae+
BHS27	2	Both	-	NotDet	NotDet (-7±3%)	P: Btre, Mhae, Psp
BHS28	2	Both	-	NotDet	NotDet (13±26%)	P: Btre, Mhae+, Pmul, Psp
BHS31	2	Both	-	NotDet	NotDet (7±16%)	P: Btre, Psp

[1] - = not done; LC = lung consolidation; CSE = cornual sinus exudate; PA = pleural adhesions; l = left lung; r = right lung.

[2] %I = Percent inhibition; NotDet = not detected; Det = detected; N = nasal passage swab; B = bronchus swab; CS = cornual sinus swab; L = lung tissue;Ð = not done.

[3] Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi;* Mhae = *Mannheimia haemolytica;* Pmul = *Pasteurella multocida;* Psp = *Pasteurella* species; + = *lktA* PCR positive; NotDet = No Pasteurellaceae detected.

https://doi.org/10.1371/journal.pone.0178707.t001

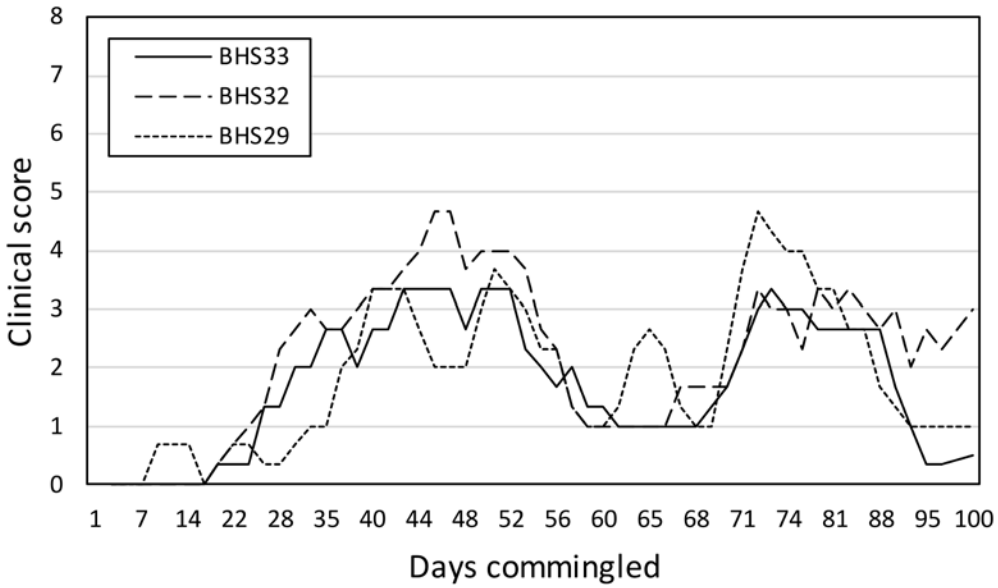

Fig 1. Clinical scores exhibited by bighorn sheep commingled with *M. ovipneumoniae* carrier domestic goats in Experiment 1. Three-day rolling average summed clinical scores based on observed anorexia, nasal discharge, cough, dyspnea, head shaking, ear paresis and weakness/incoordination. *M. ovipneumoniae* colonized DG were comingled with naïve BHS on day 0.

https://doi.org/10.1371/journal.pone.0178707.g001

none of these lambs had detectable pneumonia either grossly or histologically. The twin lambs born to group 2 (non-commingled; *M. ovipneumoniae*-negative) ewe BHS31 (BHS31L1 and BHS31L2) also died at 4 and 7 days of age, respectively, with signs of ocular, systemic and/or enteric disease. The lamb of group 2 BHS27 (BHS27L) was euthanized at 45 days of age necessitated by intractable diarrheal disease, while the lamb of BHS28 (BHS28L) survived.

Experiment 2

Group 2 DG, *M. ovipneumoniae*-free but carrying diverse pharyngeal Pasteurellaceae (Table 3), were commingled with group 2 BHS in pen #1. No respiratory disease signs were observed in either DG or BHS for >120 days during experiment 2.

Table 2. Outcomes and microbiological findings of lambs born during experiment 1.

Animal ID	Pen	Outcome[1]	*M. ovipneumoniae*[2]		*Pasteurellaceae* (*lktA*)[3]
			PCR	cELISA (% I)	
BHS29L	1	C (d6), death (d7)	Det: L N C	NotDet (0%)	P, L: Btre; C: Mhae+
BHS32L	1	C, D, death (d5)	Det: N	Det (50%)	P: Mhae+; C: Mhae+;
BHS33L	1	C, death (d7)	Det: N, B	Ind (49%)	P: Msp, Pmul; C: Mhae+; L: NotDet
BHS27L	2	D, euthanized d45)	NotDet	NotDet (2%)	NotDet
BHS28L	2	D (d54), survived	NotDet	NotDet (<4%)	P: Btre, Msp
BHS31L1	2	Death (d4)	NotDet	Not done	L: Mhae
BHS31L2	2	D, death (d10)	NotDet	NotDet (-9%)	L, C: Mhae+

[1]C = conjunctivitis, D = diarrhea.

[2]%I = Percent inhibition; Det = detected; NotDet = not detected; Ind = indeterminate result; L = lung tissue; N = nasal passage swab; C = Conjunctival swab; B = Bronchial swab

[3] Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; C = conjunctival swab; Btre = *Bibersteinia trehalosi;* Mhae = *Mannheimia haemolytica;* Pmul = *Pasteurella multocida; M*sp = *Mannheimia* species; + = *lktA* PCR positive; NotDet = No Pasteurellaceae detected.

https://doi.org/10.1371/journal.pone.0178707.t002

Table 3. Microbiological status and pathologic lesions of animals in experiments 2 and 3.

Animal ID	Experiment	Gross necropsy[1]	*M. ovipneumoniae*[2]		*Pasteurellaceae* (*lktA*)[3]
			PCR[2]	cELISA (%I)	
BHS28	Pre 2	-	NotDet	NotDet (26%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (12%)	P: Btre, Msp, Psp
	Post 3	PA (r, l)	Det: N, B	Det (79%)	P: Btre; L: Btre, Pmul
BHS28L	Pre 2	-	NotDet	NotDet (4%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (15%)	P: Msp
	Post 3	LC (5% r, 1% l)	Det: N	Det (83%)	P: Btre; L: Btre
BHS31	Pre 2	-	NotDet	NotDet (7%)	P: Btre, Psp
	Post 2 Pre 3	-	NotDet	NotDet (16%)	P: NotDet
	Post 3	No lesions seen	Det: N, B	Det (77%)	P: Btre; L: Btre, Psp
DG4	Pre 2	-	NotDet (x5)	NotDet (29%)	P: Btre, Mhae+
	Post 2 Pre 3	-	NotDet	NotDet (20%)	P: Btre
	Post 3	LC (10% r, 5% l)	Det: N, B	Det (79%)	L: Mhae
DG5	Pre 2	-	NotDet (x5)	NotDet (21%)	P: Btre, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (21%)	P: Mhae
	Post 3	LC (5% r, 5% l)	Det: N, B	Det (55%)	P: Btre; L: Mhae, Btre
DG6	Pre 2	-	NotDet (x5)	NotDet (28%)	P: Btre; Mhae+, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (27%)	P: Btre, Mhae
	Post 3	No lesions seen	Det: N, B	Det (63%)	P: Btre; L: Btre, Mhae

[1] - = not done, PA = pleural adhesions, LC = lung consolidation; l = left lung; r = right lung.

[2] %I = Percent inhibition; NotDet = not detected, Det = detected, N = nasal passage swab; B = bronchial swab.

[3] Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi;* Mhae = *Mannheimia haemolytica;* Pmul = *Pasteurella multocida; M*sp = *Mannheimia* species; Psp = *Pasteurella* species; + = *lktA* PCR positive; NotDet = No Pasteurellaceae detected by aerobic bacterial culture or by PCR.

https://doi.org/10.1371/journal.pone.0178707.t003

Experiment 3

Previously *M. ovipneumoniae*-free DG4 and DG5 began showing respiratory disease signs while still in isolation following *M. ovipneumoniae* inoculation and these signs continued after comingling in pen 1. All other pen 1 animals also developed signs of respiratory tract disease beginning shortly after re-comingling of DG4 and DG5 (Fig 2, S1 Table). The clinical respiratory disease scores of all animals in experiment 3 began diminishing after approximately 70 days of comingling. All animals were humanely euthanized for complete necropsy examinations at the end of experiment 3.

Necropsy findings

Gross necropsy examination of the bighorn sheep in experiments 1 and 3 and in the domestic goats in experiment 3 revealed limited areas of lung consolidation in most but not all animals (Tables 1 and 3). These areas were localized to the ventral tips or edges of the middle and anterior lobes, and were darker red in color and firmer on palpation than the adjacent normal lung tissues. In addition, several animals had strong fibrous adhesions between these same lung regions and the pericardial or parietal pleura, presumably representing resolved pleuritis. All animals in the study had similar histopathologic lesions that varied in severity, consisting of inflammation centered around bronchi and bronchioles and extending to include adjacent alveoli (Fig 3). Inflammation was characterized by peribronchiolar and perivascular lymphoid hyperplasia with secondary suppurative bronchiolitis and alveolar atelectasis.

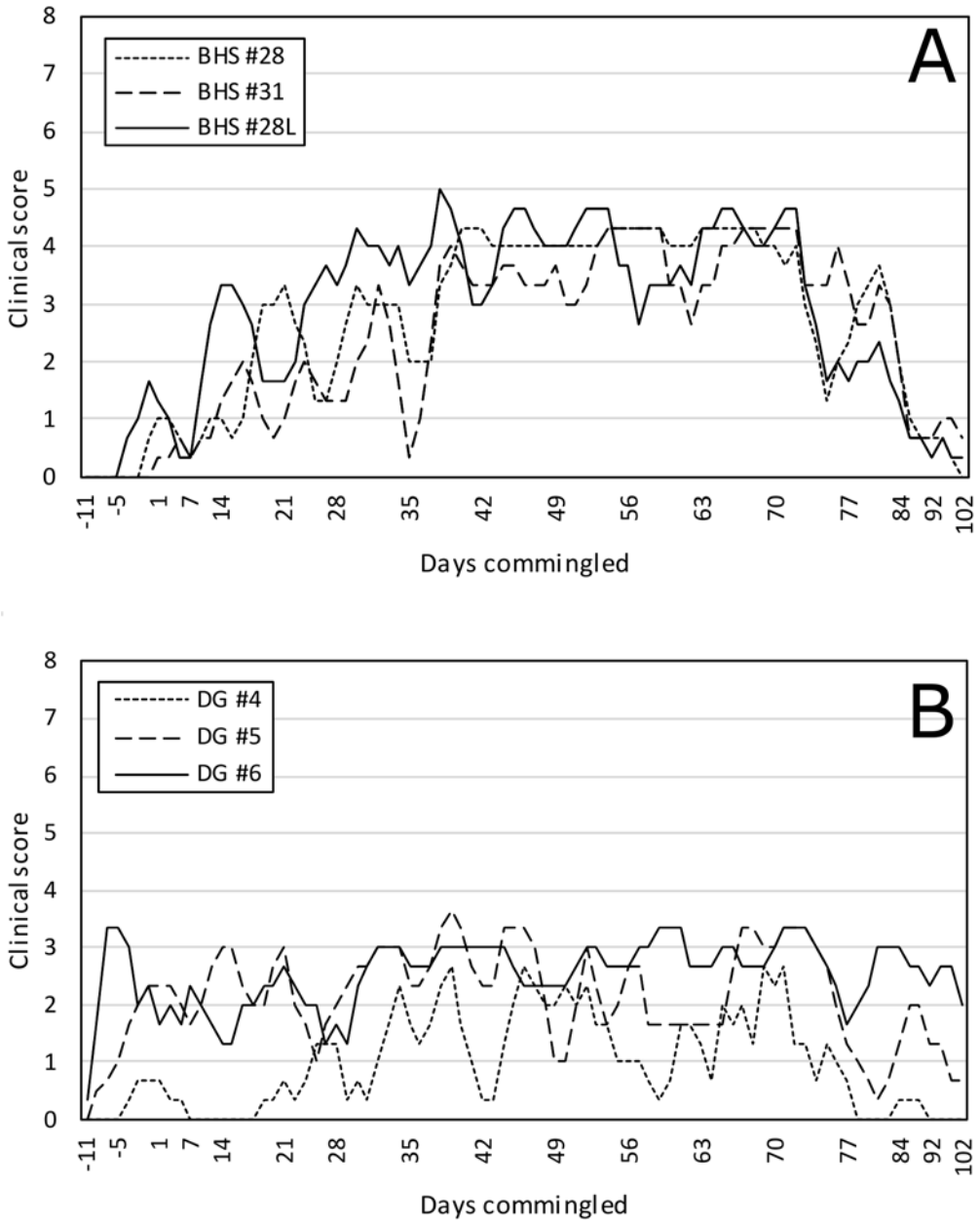

Fig 2. Clinical scores exhibited by commingled bighorn sheep and domestic goats in Experiment 3. Three-day rolling average summed clinical scores as described in Fig 1. (A) BHS; (B) DG. All BHS and DG in these experiments were naïve to *M. ovipneumoniae* prior to the experiment. DG4 and DG5 were exposed to *M. ovipneumoniae* on Day -11, and comingled with DG6 and BHS28, BHS28L and BHS31 on day 0.

https://doi.org/10.1371/journal.pone.0178707.g002

Microbiology findings

Microbiology and serology confirmed *M. ovipneumoniae* infection in all BHS only after contact with colonized DG, which resulted in seroconversion to this bacterium in both BHS and previously unexposed DG. *M. ovipneumoniae* IGS genotypes detected at necrospy in bighorn sheep or domestic goats shared identical sequences with those detected in the source goats prior to the studies. In experiment 1, a single IGS genotype was detected in all domestic goats prior to and after the experiment, and in all bighorn sheep at necropsy after the experiment. In

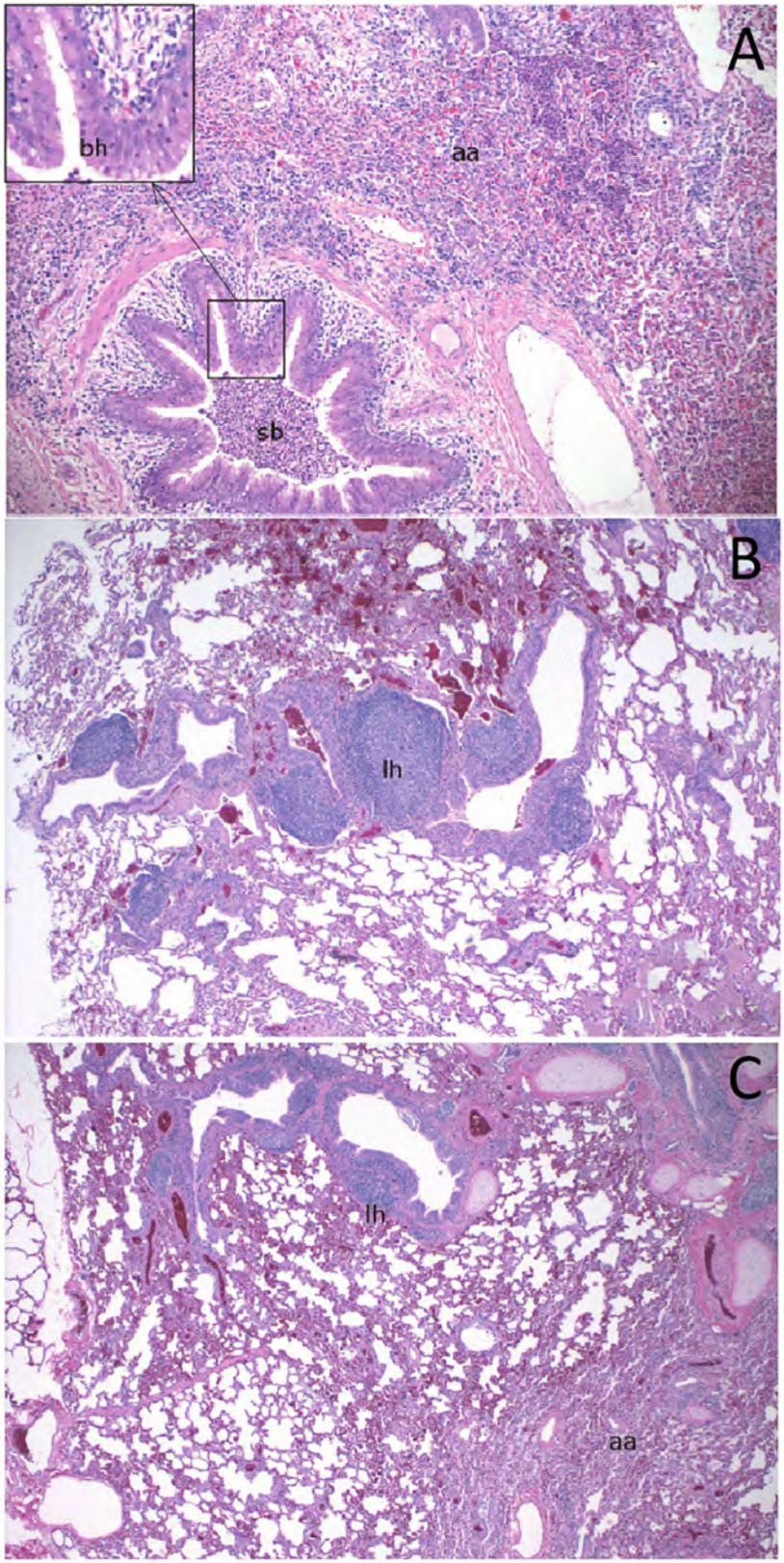

Fig 3. Representative histological lung lesions in experimental animals. Similar lesions were observed in all necropsied experimental animals. (A) BHS29 following experiment 1; (B) BHS28 following experiment 3,

and (C) DG5 following experiment 3. aa = alveolar atalectasis; bh = bronchiolar hypertrophy; lh = lymphoid hyperplasia; sb = suppurative bronchiolitis.

https://doi.org/10.1371/journal.pone.0178707.g003

experiment 2, two different IGS genotypes were detected in the domestic goat inoculum source, one of which was subsequently detected in all commingled goats and bighorn sheep, while the other strain was detected in all the domestic goats but was not detected in any of the comingled bighorn sheep. Both the bighorn sheep and domestic goat groups were colonized in the oropharynx by diverse Pasteurellaceae prior to, during, and after these experiments, and some of these bacteria were also isolated from pneumonic lung tissues of the animals at necropsy (Tables 1 and 3). These included *lktA+ M. haemolytica*, sometimes considered the most lethal Pasteurellaceae [23, 28].

Discussion

The results of this study both resemble yet substantially differ from those of previous studies of the impact of *M. ovipneumoniae* infection on initiation of pneumonia in bighorn sheep. Similar to previous studies exploring contacts with domestic sheep, comingling of *M. ovipneumoniae*-carrier domestic goats resulted in transmission of the pathogen to susceptible bighorn sheep, which then developed signs and lesions of respiratory disease [21]. However, contrary to previous studies that utilized domestic sheep carrying *M. ovipneumoniae*, the respiratory disease observed following experimental contact with domestic goats carrying *M. ovipneumoniae* was relatively mild, resulting in no fatalities. In addition, respiratory disease of comparable severity to that shown by the bighorn sheep also developed in naïve domestic goat hosts following infection, something that had not been reported in previous studies utilizing naïve domestic sheep. The lesions observed were consistent with those described in *Mycoplasma* spp. infections of sheep, goats and cattle, often called enzootic pneumonia. Enzootic pneumonia lesions are distinct from those caused by other bacterial agents of bronchopneumonia, such as *Pasteurella multocida* and *Mannheimia hemolytica*, which are characterized by widespread hemorrhage, necrosis and suppurative inflammation[13].

These contrasting results have interesting implications about the strain-specific virulence of *M. ovipneumoniae* and the specific roles of this and other bacterial pathogens in bighorn sheep respiratory disease. They also provide an area for additional research to identify management approaches for preventing new outbreaks of respiratory disease in bighorn sheep.

These results strengthen the previously documented links between exposure to *M. ovipneumoniae* and development of respiratory disease in bighorn sheep. All bighorn sheep exposed to goats carrying *M. ovipneumoniae* in experiments 1 and 3 developed signs and lesions of pneumonia, even when they had previously been exposed to the same goats in the absence of *M. ovipneumoniae*. All bighorn sheep kept in captivity under very similar conditions, but without contact with goats (experiment 1 pen 2) or in contact with *M. ovipneumoniae*-free goats (experiment 2, pen 1) did not develop signs of pneumonia. Pasteurellaceae bacteria carrying the gene encoding their principal virulence factor (*lktA* encoding leukotoxin) are another group of pathogens that have been hypothesized to play key roles in bighorn sheep pneumonia [23, 28], but both the bighorn sheep and the domestic goats used in these studies carried these pathogens prior to the experiments, and the presence of these pathogens were not clearly linked to the experimental outcomes or pathologic lesions.

Despite the consistent development of bighorn sheep pneumonia following contact with domestic goats carrying *M. ovipneumoniae*, the disease outcomes were more similar to those observed in the two previous experiments in which domestic goats were comingled with bighorn sheep and differed markedly from previous bighorn sheep-domestic sheep comingling

experiments [4, 5, 8]. Unfortunately, the *M. ovipneumoniae* carriage status of the goats used in those previous experiments was not tested and no clinical observation data were reported. The cumulative 98% mortality reported for bighorn sheep comingled with domestic sheep (88 deaths among 90 bighorn sheep in 11 experiments as reviewed in [4]) was significantly higher than either the mortality rates of those two previous goat commingling experiments (2-tailed Fisher's exact test, $P = 0.0014$ [5] and <0.0001 [8], respectively) or the two experiments reported here (each $P<0.001$). Therefore, while bighorn sheep comingled with *M. ovipneumoniae* carrier goats consistently developed respiratory disease and pneumonia, mortality was a rare outcome compared to that observed when bighorn sheep were comingling with domestic sheep carrying *M. ovipneumoniae*.

One hypothesis that may explain the differing severity of outcome of bighorn sheep infected with *M. ovipneumoniae* originating from domestic goats is that *M. ovipneumoniae* with differing virulence traits may be harbored by these two host species. Emerging evidence supports the existence of distinct clades of *M. ovipneumoniae* from these two host species [7, 14]. A naturally occurring bighorn sheep pneumonia outbreak linked to *M. ovipneumoniae* belonging to the domestic goat clade, described in [7], resulted in approximately 30% pneumonia-associated mortality in the adult bighorn sheep, similar to that reported in [5] and towards the lower end of the range of mortality rates reported in pneumonia outbreaks affecting wild bighorn sheep [3, 29±32].

Since impaired recruitment due to *M. ovipneumoniae*-associated annual lamb pneumonia mortality is recognized as a key population-limiting effect of pneumonia in bighorn sheep [11], we anticipated that the lambs born to the ewes comingled with domestic goats in these experiments would provide insights into the effects of goat-origin *M. ovipneumoniae* on lambs. In both pens, lambs experienced a similar range of diseases but no bronchopneumonia was observed in any lamb, and the deaths in pen 1 occurred prior to the age at which lamb pneumonia typically is seen. Therefore, the experiments were not informative about lamb pneumonia resulting from *M. ovipneumoniae* carried by domestic goats, although the infections experienced by the pen 1 lambs clearly documented pathogen transmission among domestic goats, bighorn ewes, and bighorn lambs. Further studies will be needed to determine the persistent effects of goat-origin *M. ovipneumoniae* on bighorn sheep lambs in the years following initial expsoure.

Conclusions and management implications: The severity of disease induced in bighorn sheep by the goat-origin *M. ovipneumoniae* strains in these experiments was relatively mild and transient. However, important questions remain: 1) Do other, more virulent strain-types exist within the goat *M. ovipneumoniae* clade? 2) Are goat-origin *M. ovipneumoniae* persistently carried by some bighorn sheep that recover from mild infections, as sheep-origin strains sometimes are? 3) If persistent carriers of goat-origin *M. ovipneumoniae* exist among bighorn ewes, are these strains subsequently transmitted to their lambs and do they induce lamb pneumonia? Answers to these questions will be relevant to the development of risk-appropriate management procedures to protect bighorn sheep from epidemic pneumonia.

Supporting information

S1 Table. Clinical scores of bighorn sheep and domestic goats.
(XLSX)

Author Contributions

Conceptualization: TEB EFC KAP WJF.

Data curation: TEB.

Formal analysis: TEB.

Funding acquisition: TEB EFC.

Investigation: TEB EFC KAP.

Methodology: TEB.

Project administration: TEB.

Resources: TEB EFC.

Supervision: TEB.

Validation: TEB.

Visualization: TEB.

Writing ± original draft: TEB.

Writing ± review & editing: TEB EFC KAP WJF.

References

1. Foreyt WJ, Jessup DA. Fatal pneumonia of bighorn sheep following association with domestic sheep. J Wildl Dis. 1982; 18(2):163±8. PMID: 7047767
2. Goodson NJ. Effects of domestic sheep grazing on bighorns sheep populations: a review. Proceedings of the Biennial Symposium of the Nothern Wild sheep and Goat Council. 1982; 3:287±313.
3. 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(2):388±403. https://doi.org/10.7589/0090-3558-44.2.388 PMID: 18436671
4. 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(1):168±72. https://doi.org/10.7589/0090-3558-48.1.168 PMID: 22247385
5. Foreyt WJ, Jenkins EJ, Appleyard GD. Transmission of lungworms (*Muellerius capillaris*) from domestic goats to bighorn sheep on common pasture. J Wildl Dis. 2009; 45(2):272±8. https://doi.org/10.7589/0090-3558-45.2.272 PMID: 19395736
6. Rudolph KM, Hunter DL, Foreyt WJ, Cassirer EF, Rimler RB, Ward ACS. Sharing of *Pasteurella* spp. between free-ranging bighorn sheep and feral goats. J Wildl Dis. 2003; 39(4):897±903. https://doi.org/10.7589/0090-3558-39.4.897 PMID: 14733287
7. Cassirer EF, Manlove K, Plowright RK, Besser TE. Evidence for strain-specific immunity to pneumonia in bighorn sheep. J Wildl Manage. 2017; 81:133±143.
8. Foreyt WJ. Effects of controlled contact exposure between healthy bighorn sheep and llamas, domestic goats, mountain goats, cattle, domestic sheep, or mouflon sheep. Proceedings of the Biennial Symposium of the Nothern Wild Sheep and Goat Council 1994; 9:7±14.
9. Besser TE, Cassirer E. F., Highland M.A., Wolff P., Justice-Allen A., Mansfield K., Davis M.A., Foreyt W. J. Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. Prev Vet Med. 2012; 108:85±93. https://doi.org/10.1016/j.prevetmed.2012.11.018 PMID: 23253148
10. 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(2):423±30. https://doi.org/10.1128/JCM.01931-07 PMID: 18057131
11. Cassirer EF, Plowright RK, Manlove KR, Cross PC, Dobson AP, Potter KA, et al. Spatio-temporal dynamics of pneumonia in bighorn sheep (*Ovis canadensis*). J Animal Ecol. 2012; 82:518±28.
12. 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(10):2593±602. https://doi.org/10.1002/ecy.1520 PMID: 27859120

13. Nicholas R, Ayling R, McAuliffe L. Mycoplasma diseases of ruminants: Disease, diagnosis and control. Cambridge MA: CABI; 2008. 256 p.
14. Maksimovic Z, De la Fe C, Amores J, Gomez-Martin A, Rifatbegovic M. Comparison of phenotypic and genotypic profiles among caprine and ovine Mycoplasma ovipneumoniae strains. Vet Rec. 2017; 180 (7):180±5. https://doi.org/10.1136/vr.103699 PMID: 27895290
15. Heinse L, Hardesty L, Harris R. Risk of Pathogen Spillover to Bighorn Sheep from Small Domestic Sheep and Goat Flocks on Private Land in Washington. Wildlife Soc B. 2016; 40(4):625±33.
16. Niang M, Rosenbusch RF, Andrews JJ, Kaeberle ML. Demonstration of a capsule on Mycoplasma ovipneumoniae. Am J Vet Res. 1998; 59(5):557±62. PMID: 9582956
17. Alley MR, Ionas G, Clarke JK. Chronic non-progressive pneumonia of sheep in New ZealandÐa review of the role of *Mycoplasma ovipneumoniae*. N Z Vet J. 1999; 47(5):155±60. https://doi.org/10.1080/00480169.1999.36135 PMID: 16032095
18. Goncalves R, Mariano I, Nunez A, Branco S, Fairfoul G, Nicholas R. Atypical non-progressive pneumonia in goats. Vet J. 2010; 183(2):219±21. https://doi.org/10.1016/j.tvjl.2008.10.005 PMID: 19596209
19. Rifatbegovic M, Maksimovic Z, Hulaj B. Mycoplasma ovipneumoniae associated with severe respiratory disease in goats. Vet Rec. 2011; 168(21):565±6.
20. Bernatowicz J, Bruning D, Cassirer EF, Harris RB, Mansfield K, Wik P. Management responses to pneumonia outbreaks in three Washington State bighorn herds: Lessons learned and questions yet unanswered. Proceedings of the Biennial Symposium of the Nothern Wild sheep and Goat Council. 2016, accepted for publication.
21. 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 (10):e110039. https://doi.org/10.1371/journal.pone.0110039 PMID: 25302992
22. Ziegler JC, Lahmers KK, Barrington GM, Parish SM, Kilzer K, Baker K, et al. Safety and immunogenicity of a *Mycoplasma ovipneumoniae* bacterin for domestic sheep (Ovis aries). Plos One. 2014; 9(4): e95698. https://doi.org/10.1371/journal.pone.0095698 PMID: 24752006
23. Lawrence PK, Shanthalingam S, Dassanayake RP, Subramaniam R, Herndon CN, Knowles DP, et al. Transmission of *Mannheimia haemolytica* from domestic sheep (*Ovis aries*) to bighorn sheep (*Ovis canadensis*): unequivocal demonstration with green fluorescent protein-tagged organisms. J Wildl Dis. 2010; 46(3):706±17; erratum, Journal of Wildlife Diseases, 46:1346. https://doi.org/10.7589/0090-3558-46.3.706 PMID: 20688676
24. McAuliffe L, Hatchell FM, Ayling RD, King AI, Nicholas RA. Detection of *Mycoplasma ovipneumoniae* in *Pasteurella*-vaccinated sheep flocks with respiratory disease in England. Vet Rec. 2003; 153(22):687± 8. PMID: 14682543
25. Roug A, Diaz-Campos D, Teitzel C, Besser TE. Comparison of two bacterial transport media for culture of tonsilar swabs from bighorn sheep and mountain goats. J Wildlife Dis. 2017; 53(1):188±192.
26. Walsh DP, Cassirer EF, Bonds MD, Brown DR, Edwards WH, Weiser GC, et al. Concordance in Diagnostic Testing for Respiratory Pathogens of Bighorn Sheep. Wildlife Soc B. 2016; 40(4):634±42.
27. Besser TE, Highland MA, 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 (3):406±14. https://doi.org/10.3201/eid1803.111554 PMID: 22377321
28. 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):1±10. https://doi.org/10.7589/2012-09-225 PMID: 24171569
29. Spraker TR, Hibler CP, Schoonveld GG, Adney WS. Pathologic changes and microorganisms found in bighorn sheep during a stress-related die-off. J Wildl Dis. 1984; 20(4):319±27. PMID: 6530718
30. Ryder TJ, Mills K. W., Bowles K. H., Thorne E. T. Effect of pneumonia on population size and lamb recruitment in Whiskey Mountain bighorn sheep. Proceedings of the Biennial Symposium of the Nothern Wild sheep and Goat Council. 1992; 8:136±46.
31. Cassirer EF, Oldenburg LE, Coggins V, Fowler P, Rudolph KM, Hunter DL, et al. Overview and preliminary analysis of Hells Canyon bighorn sheep die-off, 1995±6. Proceedings of the Biennial Symposium of the Nothern Wild sheep and Goat Council. 1996; 10:78±86.
32. Cassirer EF, Sinclair ARE. Dynamics of pneumonia in a bighorn sheep metapopulation. J Wildl Manage. 2007; 71(4):1080±8.

Journal of Wildlife Diseases, 46(4), 2010, pp. 1262–1268

A Bighorn Sheep Die-off in Southern Colorado Involving a *Pasteurellaceae* Strain that May Have Originated from Syntopic Cattle

Lisa L. Wolfe,[1,5] Brandon Diamond,[2] Terry R. Spraker,[3] Michael A. Sirochman,[1] Daniel P. Walsh,[1] Chandra M. Machin,[4] Donald J. Bade,[4] and Michael W. Miller[1] [1]Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA; [2]Colorado Division of Wildlife, 300 West New York Avenue, Gunnison, Colorado 81230, USA; [3]Colorado State University Diagnostic Laboratory, Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado 80523, USA; [4]Microbial Research, Incorporated, 2649 East Mulberry Street, Number 15, Fort Collins, Colorado 80524, USA; [5]Corresponding author (email: lisa.wolfe@state.co.us)

ABSTRACT: We investigated a pasteurellosis epizootic in free-ranging bighorn sheep (*Ovis canadensis*) wherein a *Pasteurellaceae* strain carried by syntopic cattle (*Bos taurus*) under severe winter conditions appeared to contribute to pneumonia in affected bighorns. Twenty-one moribund or dead bighorn sheep were found on the "Fossil Ridge" herd's winter range, Colorado, USA, between 13 December 2007 and 29 February 2008. Eight carcasses examined showed gross or microscopic evidence of acute to subacute fibrinous bronchopneumonia. All eight carcasses yielded at least one β-hemolytic *Mannheimia haemolytica* biogroup $1^{(\pm G)}$ strain, and seven also yielded a β-hemolytic *Bibersteinia trehalosi* biogroup 4^{CDS} strain; evidence of *Pasteurella multocida*, *Mycoplasma ovipneumoniae*, and parainfluenza 3 and bovine respiratory syncytial viruses was also detected. Isolates of β-hemolytic *Mannheimia haemolytica* biogroup 1^{G} from a bighorn carcass and a syntopic cow showed 99.5% similarity in genetic fingerprints; *B. trehalosi* biogroup 4^{CDS} isolates were ≥94.9% similar to an isolate from a nearby bighorn herd. Field and laboratory observations suggested that pneumonia in affected bighorns may have been caused by a combination of pathogens including two pathogenic *Pasteurellaceae* strains—one likely of cattle origin and one likely of bighorn origin—with infections in some cases perhaps exacerbated by other respiratory pathogens and severe weather conditions. Our and others' findings suggest that intimate interactions between wild sheep and cattle should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

Key words: *Bibersteinia trehalosi*, bighorn sheep, cattle, pneumonia, *Mannheimia haemolytica*, *Mycoplasma*, *Ovis canadensis*, *Pasteurella multocida*.

The decline of bighorn sheep (*Ovis canadensis*) abundance throughout much of western North America appears attributable to historical overharvest, habitat loss or degradation and, in large part, to epizootics caused by introduced pathogens, some of which have now become enzootic. The earliest reports of epizootics in bighorn sheep (e.g., accounts in Warren, 1910; Grinnell, 1928; Shillinger, 1937; Honess and Frost, 1942) closely followed the advent of domestic livestock grazing in bighorn habitat, suggesting that bighorn populations in some areas first may have been exposed to novel pathogens in the 1800s. More than a century later, recurring respiratory disease epizootics remain obstacles to recovering bighorn sheep populations to historic levels (Miller, 2001). Understanding and, where feasible, controlling specific risk factors that may cause or precipitate pneumonia epizootics in bighorn sheep has become an imperative of this species' conservation. Unfortunately, post hoc investigations of epizootics under field conditions rarely yield clear answers regarding source(s) of the responsible pathogen(s) and the role of potential contributing stressors like weather. Here, we describe a case wherein exposure to a pathogen carried by syntopic cattle (*Bos taurus*) under severe winter conditions may have contributed to the onset of epizootic pasteurellosis in a free-ranging bighorn herd. Our objectives are to report the findings of our field and laboratory investigations of this epizootic and to broaden conventional thinking about risk factors that may affect the health and perpetuation of North American wild sheep species.

The "Fossil Ridge" bighorn herd in

southern Colorado, USA (38°30–41′N, 106°34–48′W) was started with a translocation of 20 individuals and had grown to >60 animals by 2006 (George et al., 2009). Available range was restricted during most winters and recreation activity may have further reduced the area occupied by bighorns. As a likely consequence of limited winter range, a local Hereford breed cattle rancher reported that for about 15 yr some bighorns had come into his cattle feed lines on private land at times during fall and winter. On the basis of the belief that such interactions were not particularly risky to bighorn sheep, this behavior was not discouraged by local wildlife managers.

The winter of 2007–08 was one of the most severe in recorded history for the Gunnison Basin (Colorado Division of Wildlife, 2009), an area that included the Fossil Ridge herd's range. On the basis of data compiled at the Gunnison County Electric Association weather station for the United States National Oceanic and Atmospheric Administration, about 51 cm of heavy, wet snow fell during 6–7 December 2007, burying mountain shrub communities across the basin; below-average temperatures ranging from −7 C to −20 C precluded any appreciable snowmelt thereafter. Apparently healthy bighorn sheep were seen on traditional winter range during the week of 16 December, although no lambs from the previous summer were observed.

The epizootic at Fossil Ridge was first reported on 23 December 2007 by the local rancher, who noticed fewer bighorns in the area and subsequently found three carcasses and two sick animals nearby. Clinical signs included depression, thick nasal discharge, and dyspnea, but little coughing. One sick animal was shot; the other was found dead the next day. Subsequent field investigation on 23 and 24 December revealed additional carcasses and sick animals. In the course of discussing the situation, the rancher mentioned finding an adult female bighorn carcass 10 days earlier. He also noted that this had been a particularly bad year for respiratory disease problems in his cattle herd, perhaps because recently purchased replacement animals had "brought something in" (e.g., Frank et al., 2003); however, no previous diagnostic work had been done on the cattle herd.

Twenty-one moribund or dead bighorn sheep were found on the Fossil Ridge herd's winter range between 13 December 2007 and 29 February 2008; three additional carcass remains were found in October 2008. Eight relatively intact carcasses were necropsied; other carcasses were too scavenged, decomposed, or inaccessible to examine. Lung, tonsil, and other select tissues were submitted to the Caine Veterinary Teaching Center (CVTC; University of Idaho, Caldwell, Idaho, USA) for bacterial culture with emphasis on *Pasteurellaceae* (modified from Jaworski et al., 1998), to Microbial Research, Inc. (MRI; Fort Collins, Colorado, USA) and the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA) for *Mycoplasma* spp. culture, and to the CVTC, MRI, and the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL; Fort Collins, Colorado, USA) for PCR assays to detect *Mycoplasma* spp. DNA (Baird et al., 1999; Besser et al., 2008; D. Bade, unpubl. data; G. Weiser, pers. comm.). Antibody titers to parainfluenza 3 (PI3; recent or active infection titer ≥1:256) virus and bovine respiratory syncytial virus (BRSV; recent or active infection titer ≥1:64) were measured by virus neutralization tests at the CSUVDL. Select representative *Pasteurellaceae* isolates from carcasses and live animals (sampling detailed below) were further compared by repetitive DNA sequence genotyping by Newport Laboratories (Worthington, Minnesota, USA) using PCR and boxA1R primer (Goldberg et al., 2006). Select *Mycoplasma* spp. isolates were identified by DNA sequencing at the University of Minnesota Veterinary Diagnostic Labora-

TABLE 1. Respiratory disease agents detected from dead and surviving bighorn sheep (*Ovis canadensis*) and syntopic cattle (*Bos taurus*) during and after the December 2007 epizootic at "Fossil Ridge" in southwestern Colorado, USA. Evidence of infection or exposure came from culture data for *Pasteurellaceae*, from culture and PCR data for mycoplasmas, and from serology data for the two respiratory viruses. See text for methods and interpretation.

Agent	Bighorn sheep			Cattle
	Dead (December 2007), $n=8$	Alive (February–March 2008), $n=10$	Alive (February 2009), $n=11$	Alive (February 2008), $n=27$
Mannheimia haemolytica				
Biogroup 1 (β)[a]	5[b]	0	0	0
Biogroup 1^{G} (β)	7	0	0	1
Biogroup 1^{AG} (β)	1	0	0	0
Biogroup 3 (β)	0	0	2	0
Biogroup 3^{A}	0	0	1	0
Biogroup $16^{AG(\pm E)}$	0	0	0	20
Bibersteinia trehalosi				
Biogroup 4^{CDS} (β)	7	1	0	0
Biogroup $4^{(\pm various)}$	0	5	2	0
Biogroup $2^{(\pm various)}$	0	3	1	0
Pasteurella multocida	5	4	1	0
Mycoplasma spp.	8	3	1	15
Mycoplasma ovipneumoniae	8	sd[c]	sd	9
Mycoplasma bovirhinis	0	sd	sd	9
Bovine respiratory syncytial virus (titer ≥1:64)	2 (of 2)	2	nr[d]	8
Parainfluenza 3 virus (titer ≥1:256)	2 (of 2)	2	nr	19

[a] Isolates showed β-hemolysis on blood agar.

[b] Number of individuals positive; total sample size is shown in the column heading except where noted.

[c] Samples discarded by reference laboratory before species-specific PCR being performed.

[d] Not reported because prior vaccination confounded interpretation of titers.

tory (UMVDL; Saint Paul, Minnesota, United States). Select liver trace mineral concentrations were measured at the CSUVDL using established methods (Rosen et al., 2009) to rule out feed-associated intoxication.

All carcasses examined showed gross and microscopic evidence of acute to subacute fibrinous bronchopneumonia. The predominant microscopic lesion was severe, subacute bacterial bronchopneumonia associated with oat-shaped macrophages, edema, fibrin, and a few neutrophils filling and expanding alveolar spaces. Three dominant β-hemolytic *Pasteurellaceae* strains were recovered (Table 1). All eight carcasses yielded at least one *Mannheimia haemolytica* strain, including biogroup 1^{G} isolates from seven and biogroup 1 isolates (<83% genetic fingerprint similarity to the biogroup 1^{G} strain) from five. A *Bibersteinia trehalosi* biogroup 4^{CDS} strain also was isolated from seven carcasses. In addition, *Pasteurella multocida* (subsp. b or biotype U) was isolated from five carcasses. Lung or tonsil tissue samples from all eight bighorn carcasses tested PCR positive for *Mycoplasma ovipneumoniae* (Table 1). Serology in two cases where blood was available also suggested exposure to PI3 and BRSV. Liver tissue mineral concentrations (mean±95% confidence interval; range) for copper (146.1±78.6 parts per million [ppm] dry weight; 12.1–316 ppm), manganese (6.7±1.4 ppm; 3.8–9.7 ppm), molybdenum (3.7±1.2 ppm; 1.9–5.8 ppm), and zinc (122.7±37.3 ppm; 60.3–208 ppm)

were all within acceptable limits (Rosen et al., 2009; CSUVDL, unpubl. data; L. L. Wolfe, unpubl. data), but selenium concentrations (0.7±0.2 ppm; 0.5–1.5 ppm) were lower than reported for healthy bighorns (Rosen et al., 2009).

We captured 10 of the 11 known surviving bighorns (nine adult females and one adult male) via darting about 5 or 9 wk after the die-off was first reported. Two adult females were equipped with very-high-frequency radiocollars and the other eight animals were marked with unique plastic ear tags. We collected blood and oropharyngeal swabs and treated each animal with tulathromycin (DRAXXIN®, Pfizer Animal Health, New York, New York, USA), doramectin (DECTOMAX®, Pfizer Animal Health), and a commercial vaccine containing killed PI3, BRSV, infectious bovine rhinotracheitis virus, and bovine viral diarrhea virus (Triangle 4, Fort Dodge; Fort Dodge, Iowa, USA). In addition, we collected blood and triplicate nasopharyngeal swabs from a subset of the rancher's cattle ($n=27$) and treated all of the syntopic cattle with tulathromycin ($n=70$). We placed swabs in Port-A-Cul™ tubes (Becton, Dickinson and Company, Sparks, Maryland, USA) and hand-delivered one set to MRI and shipped the other overnight to the CVTC for culture and *Mycoplasma* spp. PCR. A third swab in brain–heart infusion broth was submitted to CSUVDL for *Mycoplasma* spp. PCR. Serum antibody titers to PI3 and BRSV were measured by serum neutralization (CSUVDL).

Nonhemolytic strains of *B. trehalosi* biogroups 2 and 4^{CDS} and *P. multocida* were the primary *Pasteurellaceae* isolated from the surviving bighorns (Table 1); *Mannheimia haemolytica* was not isolated. Nonhemolytic *M. haemolytica* biogroup $16^{AG(\pm E)}$ were the most abundant *Pasteurellaceae* isolated from cattle, although a β-hemolytic *M. haemolytica* biogroup 1^{G} also was isolated from one of the cattle (Table 1); *B. trehalosi* were not isolated from cattle. Three of the surviving bighorns and 15 of the sampled cattle were PCR positive for *Mycoplasma* spp. (Table 1); both *Mycoplama ovipneumoniae* and *Mycoplasma bovirhinis* were detected in the cattle by PCR or culture. A proportion of both the surviving bighorns and the sampled cattle had antibody titers suggesting exposure to PI3 and BRSV (Table 1).

In February 2009, surviving Fossil Ridge bighorn sheep were again baited and recaptured via drop netting. Of 11 animals captured, three (one lamb, two adult females) were unmarked and thus had not been handled in 2008. We sampled and tested bighorns as above and treated each with tulathromycin, doramectin, and two commercial vaccines, Triangle 4 and a *Mannheimia haemolytica* type A1 bacterin-toxoid (One Shot®, Pfizer Animal Health). Cultures yielded β-hemolytic *M. haemolytica* biogroup 3 (Table 1), along with nonhemolytic *B. trehalosi*, *M. haemolytica*, and *P. multocida* isolates. On the basis of numbers of non-*Pasteurellaceae* recovered, shipping and processing delays likely biased culture results. Only the lamb was PCR positive for *Mycoplasma* spp. Cattle were not resampled.

Laboratory findings linked a combination of pathogens to this epizootic. Despite sampling lags and some heterogeneity among the *Pasteurellaceae* isolated from pneumonic bighorns, a β-hemolytic, *M. haemolytica* biogroup 1^{G} isolate from a bighorn carcass showed 99.5% similarity in its genetic fingerprint to the *M. haemolytica* biogroup 1^{G} isolate from one of the syntopic cattle; moreover, these two isolates' fingerprints were ≥95.5% similar to fingerprints of other *M. haemolytica* biogroup 1^{G} isolates from temporally and geographically separate cases of domestic sheep (*Ovis aires*)-associated acute pasteurellosis in bighorns (Foreyt, 1989; George et al., 2008). These findings support the notion that domestic ruminants can harbor *Pasteurellaceae* strains

that are pathogenic in bighorn sheep. The β-hemolytic *B. trehalosi* biogroup 4^{CDS} also isolated from most pneumonic Fossil Ridge bighorns (but none of the syntopic cattle) has been recovered from several Colorado bighorn herds (Green et al., 1999; L. L. Wolfe and M. W. Miller, unpubl. data); *B. trehalosi* biogroup 4^{CDS} isolates from both dead and surviving Fossil Ridge bighorns were ≥94.9% similar by genetic fingerprinting to isolates from the nearby Taylor River bighorn herd where this strain (called "ribotype E_{CO}" elsewhere; Green et al., 1999) has been enzootic since at least the early 1990s (M. W. Miller and L. L. Wolfe, unpubl. data). These findings support the notion that enzootic *Pasteurellaceae* also can contribute to pneumonia during epizootics in bighorn sheep. In addition to *Pasteurellaceae*, both bighorns and syntopic cattle showed evidence of exposure to *Mycoplasma ovipneumoniae* (most likely of bighorn origin), PI3, and BRSV.

On the basis of findings from necropsy and live animal sampling, we believe that this pneumonia epizootic was caused by a combination of pathogens including two or more pathogenic strains of *Pasteurellaceae*—a *Mannheimia haemolytica* strain most likely of cattle origin and a *B. trehalosi* strain most likely of bighorn origin—with some cases perhaps exacerbated by exposure to *Mycoplasma* spp. and viruses of cattle or bighorn origin. Despite what we believe to be compelling support for this explanation, however, we recognize that identifying the true cause(s) of this and other pasteurellosis epizootics in bighorn sheep retrospectively under field conditions cannot be done with certainty. For example, interpretation of culture data is complicated by the heterogeneity and dynamics of *Pasteurellaceae* in bighorns and in domestic sheep and cattle (Miller et al., 1997; Jaworski et al., 1998; Miller, 2001; Safaee et al., 2006; Kelley et al., 2007; George et al., 2008; Tomassini et al., 2009), and is further confounded by influences of sample handling and laboratory methods (Safaee et al., 2006; George et al., 2008; Dassanayake et al., 2009a; L. L. Wolfe, unpubl. data) and the potential for pathogenicity to change within strains via horizontal transfer of the gene encoding leukotoxin (Kelley et al., 2007). In addition to the pathogens we detected, weather conditions may have contributed at Fossil Ridge either as a stressor on bighorns or cattle, or simply by increasing interactions between bighorns and cattle (Fig. 1). Notably, however, we did not observe epizootic pasteurellosis in a bighorn herd wintering in the nearby Taylor River drainage despite equally severe winter conditions and the presence of several pathogens also present in the Fossil Ridge herd (β-hemolytic *B. trehalosi* biogroup 4^{CDS}, PI3, BRSV, *Mycoplasma ovipneumoniae*; L. L. Wolfe, unpubl. data), suggesting that the presence of *Mannheimia haemolytica* or *Mycoplasma bovirhinis* in syntopic cattle may have helped trigger the Fossil Ridge epizootic.

FIGURE 1. The intensity and duration of interactions between bighorn sheep and cattle on feed lines during December 2007 may have contributed to the apparent exchange of respiratory pathogens associated with a pasteurellosis epizootic in the "Fossil Ridge" bighorn herd that resided in southern Colorado, USA.

Segregating wild sheep from domestic sheep has long been recognized as important to preventing epizootics in bighorn sheep (Warren, 1910; Shillinger, 1937; Foreyt and Jessup, 1982). Thus far, similar emphasis has not been placed on prevent-

ing interactions between cattle and bighorn sheep, most likely because species differences and a tendency toward interspecies avoidance are thought to help minimize opportunities for pathogen exchange (Foreyt and Lagerquist, 1996). However, the similarities between *Pasteurellaceae* and other respiratory pathogens of cattle and domestic sheep suggest similar adverse consequences to bighorn sheep if pathogen transmission were to occur between cattle and bighorns (Onderka et al., 1988; Singer et al., 2000). Such consequences have been demonstrated experimentally: five of eight bighorns died within 4 days of receiving intradermal injections of a cattle vaccine containing attenuated, live *Mannheimia haemolytica* (Onderka et al., 1988), four bighorns died within 2 days after intratracheal inoculation with *M. haemolytica* isolated from cattle (Dassanayake et al., 2009b), and one of five captive bighorns died 6 days after being copastured with Holstein calves (Foreyt and Lagerquist, 1996). We conclude from our findings, combined with other published observations, that intimate interactions between wild sheep and cattle (e.g., shared feed lines or troughs) also should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

Our work was funded by the Colorado Division of Wildlife and the Rocky Mountain Bighorn Society (RMBS). We thank N. and D. Means for property access and assistance with field capture and for their love of wildlife. We also thank M. Thorpe, J. Wenum, and others for field assistance; I. LeVan, K. Griffin, K. Fox, L. Baeten, and others for assistance with necropsies and sample handling; G. Weiser and L. Lasher at CVTC and A. Hinson, T. Gillette, A. Richecky, T. Morine, and S. Stabler at MRI for technical assistance with bacterial culture, PCR, and isolate identification; R. Bey, B. Hause, R. Shirbroun, and others at Newport Laboratories for assistance with *Pasteurellaceae* strain characterizations; S. Oliveira and B. Schloeder at the UMVDL for *Mycoplasma* spp. sequencing; and R. Kahn for seeking and D. Larkin (RMBS) for facilitating emergency funding to support our field work. G. Weiser, D. Martin, and anonymous reviewers provided helpful comments on earlier drafts.

LITERATURE CITED

Baird, S. C., J. Carman, R. P. Dinsmore, R. L. Walker, and J. K. Collins. 1999. Detection and identification of *Mycoplasma* from bovine mastitis infections using a nested polymerase chain reaction. Journal of Veterinary Diagnostic Investigation 11: 432–435.

Besser, T. E., K. A. Potter, E. F. Cassirer, J. Vanderschalie, A. Fischer, D. P. Knowles, D. R. Herndon, F. R. Rurangirwa, G. C. Weiser, and S. Srikumaran. 2008. Association of *Mycoplasma ovipneumoniae* infection with population-limiting respiratory disease in free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). Journal of Clinical Microbiology 46: 423–430.

Colorado Division of Wildlife. 2009. Gunnison winter feeding operations guide winter 2007–2008. Final February 11, 2009 revised April 24, 2009. Colorado Division of Wildlife, Denver, Colorado, 101 pp.

Dassanayake, R. P., D. R. Call, A. A. Sawant, N. C. Casavant, G. C. Weiser, D. P. Knowles, and S. Srikumaran. 2009a. *Bibersteinia trehalosi* inhibits its growth of *Mannheimia haemolytica* by a proximity-dependent mechanism. Applied Environmental Microbiology, published online ahead of print on 28 December 2009. doi:10.1128/AEM.02086-09.

———, S. Shanthalingam, C. N. Herndon, P. K. Lawrence, E. F. Cassirer, K. A. Potter, W. J. Foreyt, K. D. Clinkenbeard, and S. Srikumaran. 2009b. *Mannheimia haemolytica* serotype A1 exhibits differential pathogenicity in two related species, *Ovis canadensis* and *Ovis aries*. Veterinary Microbiology 133: 366–371.

Foreyt, W. J. 1989. Fatal *Pasteurella haemolytica* pneumonia in bighorn sheep after direct contact with clinically normal domestic sheep. American Journal of Veterinary Research 50: 341–344.

———, and D. A. Jessup. 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. Journal of Wildlife Diseases 18: 163–168.

———, and J. E. Lagerquist. 1996. Experimental contact of bighorn sheep (*Ovis canadensis*) with horses and cattle, and comparison of neutrophil sensitivity to *Pasteurella haemolytica* cytotoxins. Journal of Wildlife Diseases 32: 594–602.

FRANK, G. H., R. E. BRIGGS, G. C. DUFF, AND H. S. HURD. 2003. Effect of intranasal exposure to leukotoxin-deficient *Mannheimia haemolytica* at the time of arrival at the feedyard on subsequent isolation of *M. haemolytica* from nasal secretions of calves. American Journal of Veterinary Research 64: 580–585.

GEORGE, J. L., D. J. MARTIN, P. M. LUKACS, AND M. W. MILLER. 2008. Epidemic pasteurellosis in a bighorn sheep population coinciding with the appearance of a domestic sheep. Journal of Wildlife Diseases 44: 388–403.

———, R. KAHN, M. W. MILLER, AND B. WATKINS. 2009. Colorado bighorn sheep management plan 2009–2019. Colorado Division of Wildlife Special Report Number 81, Colorado Division of Wildlife, Department of Natural Resources, Denver, Colorado, 88 pp.

GOLDBERG, T. L., T. R. GILLESPIE, AND R. S. SINGER. 2006. Optimization of analytical parameters for inferring relationships among *Escherichia coli* isolates from repetitive-element PCR by maximizing correspondence with multilocus sequence typing data. Applied and Environmental Microbiology 72: 6049–6052.

GREEN, A. L., N. M. DUTEAU, M. W. MILLER, J. TRIANTIS, AND M. D. SALMAN. 1999. Polymerase chain reaction techniques for differentiating cytotoxic and noncytotoxic *Pasteurella trehalosi* from Rocky Mountain bighorn sheep. American Journal of Veterinary Research 60: 583–588.

GRINNELL, G. B. 1928. Mountain sheep. Journal of Mammalogy 9: 1–9.

HONESS, R. F., AND N. M. FROST. 1942. A Wyoming bighorn sheep study. Wyoming Game and Fish Department Cheyenne Bulletin Number 1, Cheyenne, Wyoming, 126 pp.

JAWORSKI, M. D., D. L. HUNTER, AND A. C. S. WARD. 1998. Biovariants of isolates of *Pasteurella* from domestic and wild ruminants. Journal of Veterinary Diagnostic Investigation 10: 49–55.

KELLEY, S. T., E. F. CASSIRER, G. C. WEISER, AND S. SAFAEE. 2007. Phylogenetic diversity of Pasteurellaceae and horizontal gene transfer of leukotoxin in wild and domestic sheep. Infection, Genetics and Evolution 7: 13–23.

MILLER, M. W. 2001. Pasteurellosis. *In* Infectious diseases of wild mammals. 3rd Edition, E. S. Williams and I. K. Barker (eds.). Iowa State University Press, Ames, Iowa. pp. 330–339.

———, J. A. CONLON, H. J. MCNEIL, J. M. BULGIN, AND A. C. S. WARD. 1997. Evaluation of a multivalent *Pasteurella haemolytica* vaccine in bighorn sheep: Safety and serologic responses. Journal of Wildlife Diseases 33: 738–748.

ONDERKA, D. K., S. A. RAWLUK, AND W. D. WISHART. 1988. Susceptibility of Rocky Mountain bighorn sheep and domestic sheep to pneumonia induced by bighorn and domestic livestock strains of *Pasteurella haemolytica*. Canadian Journal of Veterinary Research 52: 439–444.

ROSEN, L. E., D. P. WALSH, L. L. WOLFE, C. L. BEDWELL, AND M. W. MILLER. 2009. Effects of selenium supplementation and sample storage time on blood indices of selenium status in bighorn sheep. Journal of Wildlife Diseases 45: 795–801.

SAFAEE, S., G. C. WEISER, E. F. CASSIRER, R. R. RAMEY, AND S. T. KELLEY. 2006. Microbial diversity in bighorn sheep revealed by culture-independent methods. Journal of Wildlife Diseases 42: 545–555.

SHILLINGER, J. E. 1937. Disease relationship of domestic stock and wildlife. *In* Transactions of the Second North American Wildlife Conference. American Wildlife Institute, Washington, DC, pp. 298–302.

SINGER, F. J., C. M. PAPOUCHIS, AND K. K. SYMONDS. 2000. Translocations as a tool for restoring populations of bighorn sheep. Restoration Ecology 8: 6–13.

TOMASSINI, L., B. GONZALES, G. C. WEISER, AND W. SISCHO. 2009. An ecologic study comparing distribution of *Pasteurella trehalosi* and *Mannheimia haemolytica* between Sierra Nevada bighorn sheep, White Mountain bighorn sheep, and domestic sheep. Journal of Wildlife Diseases 45: 930–940.

WARREN, E. R. 1910. The mountain sheep. *In* The mammals of Colorado: An account of the several species found within the boundaries of the State, together with a record of their habits and of their distribution. G. P. Putnam's Sons, Knickerbocker Press, New York, New York, pp. 9–12.

Submitted for publication 30 November 2009.
Accepted 13 April 2010.