Journal of Zoo and Wildlife Medicine 38(4): 548–558, 2007

MICROORGANISMS ASSOCIATED WITH A PNEUMONIC EPIZOOTIC IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*)

Karen M. Rudolph, B.A., Ph.D., David L. Hunter, B.S., D.V.M., Richard B. Rimler, M.S., Ph.D., E. Frances Cassirer, M.S., Ph.D., William J. Foreyt, M.S., Ph.D., Walter J. DeLong, B.S., M.S., Glen C. Weiser, M.S., Ph.D., and Alton C. S. Ward, M.S., Ph.D.

Abstract: A comprehensive study of a pneumonic epizootic was initiated when the first signs of disease were noted in a metapopulation of bighorn sheep inhabiting Hells Canyon, bordering Idaho, Oregon, and Washington. A total of 92 bighorn sheep were tested for etiologic agents during the following 6-mo study period. The study population included bighorn sheep believed to be the subpopulation in which disease was first noted, and these sheep were translocated to a holding facility in an effort to contain the disease (group A_1, $n = 72$); bighorn sheep in other subpopulations (group A_2) with evidence of clinical disease were captured, sampled, given antibiotics, and released ($n = 8$) and those that were found dead were necropsied ($n = 12$). Samples, including oropharyngeal and nasal swabs, and lung and liver tissue were collected from the bighorn sheep identified above. Tissue was collected at necropsy from 60 group A_1 bighorn sheep that died following translocation, and samples were cultured for bacteria and viruses. Blood samples were tested for antibodies against known respiratory viruses, and histopathology was conducted on tissue samples. The major cause of death in both group A_1 and group A_2 bighorn sheep was a rapidly developing fibrinous bronchopneumonia. Multiple biovariants of *Pasteurella* were isolated from oropharyngeal and nasal samples from both groups, and *Mycoplasma ovipneumonia* was isolated from five group A_1 oropharyngeal samples. Organisms isolated from lung tissue included *Pasteurella multocida multocida a* and *Pasteurella trehalosi*, both of which differentiated into multiple strains by restriction enzyme analysis, and parainfluenza-3 virus (PI-3). Paired serum samples revealed >fourfold increases in titers against PI-3 and bovine respiratory syncytial viruses. It was concluded that this epizootic resulted from a complex of factors including multiple potential respiratory pathogens, none of which were identified as a primary pathogen, and possible stress factors.

Key words: Bighorn sheep, *Ovis canadensis canadensis*, *Mycoplasma* spp., parainfluenza-3 virus, *Pasteurella multocida* spp., respiratory syncytial virus.

INTRODUCTION

Pneumonic epizootics have been important in the historical decline of bighorn sheep populations in the United States,[9] and they pose a serious challenge to restoration efforts.[34] The majority of the epizootics were evaluated only after numerous deaths occurred rather than at the first signs of illness or while disease outbreaks were in progress.[16,38,47,54] Varied species, colony types, and serotypes of *Pasteurella* have been incriminated as etiologic agents associated with bighorn sheep epizootics.[18,35,36,38,40,51] Although factors, including heavy parasitism and viral infections, which can predispose animals to pneumonic pasteurellosis, have been associated with some bighorn sheep die-offs,[32] the etiology of most epizootics has not been clearly identified.[34] In many instances, deaths can result from compounding effects of primary pathogens, secondary infections, and predation resulting from disease-associated weakness. When studies involve only dead animals or live animals after the initial stage of an epizootic, primary pathogens may no longer be present or may be present in such low numbers that they are rarely detectable. Evaluation of blood serum from dead or surviving animals for antibodies against potential respiratory pathogens can provide valuable information regarding previous exposure to those organisms; however, inability to obtain both acute and convalescent blood samples frequently results in inconclusive evidence of viral association with disease. A better understand-

From the Idaho Department of Fish and Game, 600 South Walnut, Boise, Idaho 83707, USA (Rudolph); Turner Enterprises, 1123 Research Drive, Bozeman, Montana 59718, USA (Hunter); United States Department of Agriculture, Agricultural Research Service, National Animal Disease Laboratory, P.O. Box 70, Ames, Iowa 50010, USA (Rimler, deceased); Idaho Department of Fish and Game, 3316 16th Street, Lewiston, Idaho 83501, USA (Cassirer); Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164, USA (Foreyt); Idaho State Health Laboratory, 2220 Old Penitentiary Road, Boise, Idaho 83712, USA (DeLong); University of Idaho Caine Veterinary Teaching Center, 1020 East Homedale Road, Caldwell, Idaho 83607, USA (Weiser, Ward). Correspondence should be directed to Dr. Rudolph (krudolph@idfg.idaho.gov).

ing of the etiology of bighorn sheep pneumonic epizootics is crucial for restoration efforts to be successful and for healthy bighorn sheep populations to be maintained.

A unique opportunity to collect samples from a bighorn sheep metapopulation occurred in the early phase of and during a pneumonic epizootic. Following is a comprehensive report of data collected during that epizootic, which implicates multiple etiologic agents.

MATERIALS AND METHODS

Bighorn sheep populations

During November and December 1995, multiple deaths resulting from respiratory disease in the Hells Canyon bighorn sheep metapopulation were detected.[12,46] Based on a multiagency decision, 72 bighorn sheep, believed to comprise the majority of remaining live animals in the Black Butte subpopulation in which disease was first detected (group A_1), were captured by helicopter net-gunning operations on 2 December (n = 25), 3 December (n = 33), and 12 December (n = 14) 1995 in an attempt to prevent spread of disease to bighorn sheep in other subpopulations. The clinical condition of each bighorn sheep was noted at capture; 40 had evidence of *Psoroptes* spp. infestation (scabies), four had horn fractures, three were in thin body condition, three were noted to have nasal discharge, and two had clinical pneumonia. All animals were sampled, then given oxytetracycline, penicillin, and ivermectin and were immediately transported by truck and trailer to the Idaho Department of Fish and Game Wildlife Health Laboratory (WHL) near Caldwell, Idaho (USA), where sheep captured on each date were placed in separate 0.1-ha or 0.15-ha pens. Strict adherence to isolation was maintained to prevent exposure to other ruminant species, and a second injection of ivermectin was given 1 wk following capture. Daily observations were made using binoculars to minimize stress, and handling of individual animals was done only as warranted. When respiratory disease was indicated by excessive nasal discharge and coughing and/or lethargy was observed in bighorn sheep following capture, they were treated with additional oxytetracycline or other antibiotics (gentamicin, ceftiofur, tylosin, amoxicillin, and/or tilmicosin). Necropsies were performed on bighorn sheep that died at WHL.

Free-ranging bighorn sheep in adjacent Hells Canyon subpopulations were also monitored for signs of respiratory disease between 2 December 1995 and 16 April 1996.[12] Samples were collected from 20 bighorn sheep (group A_2), including eight with evidence of respiratory disease that were captured by net gunning or were darted from the ground, sampled, treated with antibiotics, and released on site; we also collected samples from 12 bighorn sheep that were found dead or agonal and that were necropsied in the field.

Collection of samples

Oropharyngeal (OP) swab samples were collected for bacterial culture using Accu-Culshure systems (Accu-Med Corporation, Pleasantville, New York 10570-2982, USA) from 70 group A_1 and eight group A_2 bighorn sheep at the time of capture and from 10 of 12 group A_2 bighorn sheep necropsied in the field. Nasal (NA) samples were also collected at that time for bacterial culture from 59 group A_1 and five group A_2 live bighorn sheep using swab systems with Amies modified transport medium (Precision Dynamics Corporation, San Fernando, California 91340-3490, USA). Rayon-tipped swabs (Viral Culturette, Becton Dickinson, Sparks, Maryland 21152, USA) were used to collect OP samples for viral culture from 17 group A_1 bighorn sheep upon their arrival at WHL on 2 December (n = 2), 3 December (n = 1), and 12 December (n = 14) 1995 and from one live captured group A_2 bighorn sheep on 5 February 1996. Lung tissue was collected for bacterial culture from all 60 group A_1 bighorn sheep that died between 19 December 1995 and 25 March 1996 and from 11 group A_2 bighorn sheep during necropsy. Lung tissue from nine group A_1 animals was submitted for viral culture. Liver samples collected from 44 group A_1 and three dead group A_2 bighorn sheep were also submitted for bacterial culture. Portions of tissues from 44 group A_1 and four group A_2 bighorn sheep were fixed in 10% buffered formalin for histologic evaluation.

Laboratory test procedures

Samples collected from three live and four necropsied group A_2 bighorn sheep were submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL) in Pullman, Washington (USA) and cultured for bacteria using standard procedures.[10] All other samples were processed at the University of Idaho Caine Veterinary Teaching Center (CVTC) at Caldwell, Idaho (USA), where they were inoculated within 24 hr following collection onto Columbia blood agar (Becton Dickinson) with 5% sheep blood and a selective Columbia blood agar with 5% bovine blood, which contained antibiotics that inhibit most non-Pasteurellaceae organisms and enhance isolation of Pasteurellaceae.[30]

All cultures were incubated at 35–37°C with 10% added CO_2. Cultures conducted at CVTC were evaluated after 24 and 48 hr of incubation, and representative colonies were identified. Those with characteristics of *Pasteurella* isolated at CVTC and those isolated at WADDL and transferred to CVTC were characterized further using biochemical utilization tests for biovariant identification.[29] Non-Pasteurellaceae isolates were identified as described previously.[42]

A group of 22 *Pasteurella multocida* isolates was submitted to the National Animal Disease Center (NADC; Ames, Iowa, USA) for determination of capsular and somatic antigens[43] and DNA restriction enzyme analysis (REA) using *Hha*I.[59] This group of isolates included 16 from group A_1 samples—NA (n = 1), lung (n = 9), liver (n = 4), and lymph node (n = 2)—and four from live captured group A_2—NA (n = 3) and OP (n = 1)—and two from lung tissue of necropsied group A_2 bighorn.

The REA profiles were also determined for six *Pasteurella trehalosi* biovariant 2 and 10 biovariant 2^B isolates using *Hha*I, as previously described,[29] to determine if a specific strain was associated with disease. Biovariant 2 isolates were representative of both bighorn sheep groups (group A_1, n = 1 OP isolate and group A_2, n = 4 OP isolates and n = 1 NA isolate). Similarly, biovariant 2^B isolates were from bighorn sheep group A_1 (n = 2 OP isolates) and group A_2 (OP, n = 5 isolates; NA, n = 1 isolate; and lung, n = 2 isolates).

Swab samples collected at the time of capture of group A_1 bighorn sheep, lung tissue from 36 of those animals that died in captivity, and lung samples from two group A_2 bighorn sheep were inoculated onto modified Hayflick's agar[25] for detection of *Mycoplasma* spp. Hayflick's agar plates were incubated at 35°C to 37°C with 10% added CO_2 and were examined for colonies characteristic of *Mycoplasma* spp. up to 30 days after inoculation. All isolates confirmed to be *Mycoplasma* by sensitivity to digitonin were propagated in Hayflick's broth, frozen at −70°C, and sent to the University of California, Davis (Davis, California, USA) for speciation by standard procedures.[24]

Swab samples submitted for viral culture were placed in 2 ml of Hank's balanced salt solution (HBSS) (Invitrogen/Gibco/BRL Life Technologies, Inc., Grand Island, New York, 14072, USA) supplemented with 0.5% gelatin, 1,000 U penicillin, 1 mg streptomycin, and 10 μg amphotericin B/ml, which comprised an antibiotic solution (HBSS-A). Lung samples were minced and then ground in HBSS-A solution (20% w/v) with TenBroeck tissue grinders (Fisher Scientific, Houston, Texas 77251, USA). Tissue suspensions were clarified by centrifugation (1,000 g, 15 min), and the supernatant fluid was frozen (−70°C) until virus isolation procedures were conducted. Supernatants were thawed and 200 μl of fluid was inoculated into 24-well microplates (Invitrogen/Gibco/BRL Life Technologies) containing circular (12-mm diameter) coverslips covered with confluent monolayers of bovine turbinate cells. Following a 1-hr absorption period, monolayers were rinsed with HBSS-A and overlaid with minimum essential medium (Invitrogen/Gibco/BRL Life Technologies) containing 2% fetal bovine serum (Hyclone Laboratories, Logan, Utah 84321, USA). The cultures were examined daily for cytopathic effects. Cultures were subpassaged at least three times at 7-day intervals. On the third passage, the monolayers were stained by indirect fluorescent antibody technique using monoclonal antibodies to bovine respiratory syncytial (BRS), bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), ovine progressive pneumonia, and parainfluenza-3 (PI-3) viruses (Chemicon International Incorporated, Temecula, California 92590, USA). Positive samples were tested for chloroform sensitivity to aid in identification. In addition, lung fluid samples were tested for BRS virus antigen with a commercial enzyme immunoassay kit (Abbott Laboratories, Abbott Park, Illinois 60064, USA).

Blood samples were collected for serologic evaluation from 34 group A_1 bighorn sheep at the time of capture and from four live and two dead group A_2 bighorn sheep. A second blood sample was collected from 21 of the same group A_1 bighorn sheep 9 to 19 days following capture. Serum samples were tested at the Idaho State Bureau of Animal Health Laboratories, Boise, Idaho (USA) (n = 38) and WADDL (n = 2) for antibodies against viruses associated with respiratory disease in domestic ruminants, including BRS, BVD, IBR, and PI-3 viruses.

Formalin-fixed tissues samples were embedded, sectioned at 5–6 μm, stained with hematoxylin and eosin, and examined microscopically.

RESULTS

Bacterial isolation and characterization

Pasteurella/Mannheimia spp., hereafter referred to as *Pasteurella* spp., were isolated from all 70 OP and 30 NA swab samples collected at the time of capture and from 46 of 60 lung samples collected from group A_1 bighorn sheep at necropsy (Table 1). *Pasteurella* spp. were also isolated from six of eight OP samples from group A_2, seven of 10 OP, and

Table 1. *Pasteurella* spp. isolated from oropharyngeal (OP) and lung (LG) samples collected from bighorn sheep in Hells Canyon metapopulation during 1995–1996 epizootic.

	Biovariants[a] of *Pasteurella* spp. isolated from bighorn sheep groups[b]				
	Group A_1		Group A_2		
			Captured/released	Necropsied in field	
Pasteurella spp.	At capture OP (n = 70)[c]	At postmortem LG (n = 60)	OP (n = 8)[c]	OP (n = 10)	LG (n = 11)
P. haemolytica	3^{α} (1), $3^{\alpha BE}$ (2), 3^{BEX} (1), 3^{CDS} (1)	0	3^{α} (1)	0	0
	8 (1)	0	0	0	0
	$9^{\alpha\beta B}$ (1)	0	0	0	0
	10^{B} (1)	0	0	10^{B}	0
	$11^{\alpha\beta CDS}$ (6), $11^{\alpha\beta CDES}$ (1)	0	0	0	0
	$16^{\alpha E}$ (7)	0	0	0	0
	U^{β} (4), $U^{\alpha\beta}$ (5), $U^{\alpha\beta B}$ (9), $U^{\beta B}$ (2)	0	U^{β} (2)	0	0
P. trehalosi	2 (1), 2^{B} (65), 2^{BCD} (1)	2^{B} (8)[d]	2 (3), 2^{B} (2)	2 (1), 2^{B} (7)	2 (1), 2^{B} (2)
P. multocida	*multocida a* (5)	*multocida a* (32)	0	0	0
	multocida b (0)	*multocida b* (4)	*multocida b* (1)	0	*multocida b* (2)
	gallicida (0)	*gallicida* (7)	0	0	0
	biotype U^{6} (1)	biotype U^{6} (5)	0	0	0
	unspeciated (0)	unspeciated (3)	0	0	0

[a] Biovariants were differentiated by the procedure of Jaworski et al.[29] Numbers in parentheses following listed biovariants indicate the numbers of bighorn sheep from which that organism was isolated.

[b] Group A_1: Black Butte population in which respiratory disease was first detected; captured in attempt to prevent spread of disease to additional populations in the Hells Canyon metapopulation. Group A_2: free-ranging bighorn sheep with evidence of respiratory disease, which were captured, treated with antibiotics, and released on site in Idaho (n = 1) and Oregon (n = 7), and bighorn sheep found dead or agonal in Idaho (n = 1), Oregon (n = 9), and Washington (n = 2), which were necropsied for and samples collected for diagnostic evaluation.

[c] *Pasteurella* spp. isolated from group A_1 nasal (NA) samples: *P. haemolytica* biovariants $9^{\alpha EG}$ (n = 1) and U (n = 1), *P. trehalosi* biovariants 2^{B} (n = 21) and 4 (n = 1), *P. multocida multocida a* (n = 6), *P. multocida* U^{6} (n = 1); from group A_2 NA samples: *P. haemolytica* biovariant $16^{\alpha\beta}$ (n = 1), *P. trehalosi* biovariants 2 (n = 2) and 2^{B} (n = 4), and *P. multocida multocida a* (n = 1), and *P. multocida multocida b* (n = 3).

[d] *Pasteurella multocida* strains were also isolated from six of eight lung samples from which *P. trehalosi* biovariant 2^{B} was isolated.

five of 11 lung samples collected from this group at necropsy. The greatest diversity of isolates was from OP samples collected from group A_1 bighorn sheep, representing free-ranging animals, at the time of capture. These isolates were differentiated into 19 biovariant groups, including *Pasteurella haemolytica*, *P. trehalosi*, *P. multocida multocida a*, and a *P. multocida* biotype, U^{6}. The *Pasteurella* spp. isolated from group A_2 OP samples were identified in three *P. haemolytica* and two *P. trehalosi* biovariants and *P. multocida multocida b*. The predominant group of organisms isolated from OP samples from group A_1 and A_2 was *P. trehalosi* biovariant 2^{B}. In contrast, the majority of organisms isolated from lung tissue of group A_1 bighorn sheep were identified as *P. multocida*, with *P. multocida multocida a* most frequently isolated. In addition, *P. multocida multocida b*, *P. multocida gallicida*, and *P. multocida* biotype U^{6} were isolated from A_1 lung samples. *Pasteurella multocida multocida b* and *P. trehalosi* 2 and 2^{B} were isolated from group A_2 lung samples. Relatively few *Pasteurella* spp. organisms were isolated from NA samples. *Pasteurella multocida* strains were also isolated from 11 of 44 group A_1 liver samples, alone (n = 7) and in combination with other bacteria (n = 4). Bacteria isolated from group A_1 and group A_2 lung and liver samples in addition to *Pasteurella* spp. are presented in Table 2. *Pasteurella* spp. were not isolated from any of the group A_2 liver samples.

Restriction enzyme analysis of isolates

Of the 22 *P. multocida* isolates submitted to NADC for capsular, somatic antigen typing and REA, nine had capsular type A and 13 were acapsular. Somatic antigens detected were 1 (n = 6); 2 (n = 1); 2, 5 (n = 2); 3 (n = 1); 3, 4 (n = 1); and 5 (n = 1). Ten of the isolates did not react with available somatic typing sera. REA of the 22 *P. multocida* isolates revealed six visually different profiles identified as PM-1 through PM-6. Isolates with PM-1 and PM-3 REA profiles were cultured from both group A_1 and group A_2 bighorn sheep, while isolates with PM-2 and PM-4 REA profiles were cultured from group A_1 bighorn sheep, and

Table 2. Bacteria in addition to *Pasteurella* and *Mycoplasma* species isolated from tissue samples collected from bighorn sheep that died during the 1995–96 Hells Canyon epizootic.[a]

	Group A_1		Group A_2	
	LG[b] (n = 60)	LV (n = 44)	LG (n = 6)	LV (n = 3)
Gram-negative bacteria				
Acinetobacter spp.	3	0	0	0
Enterobacter spp.	0	0	1	1
Escherichia coli	12	25	0	0
Moraxella spp.	5	0	0	0
Proteus spp.	9	7	0	0
Pseudomonas spp.	18	3	0	0
Unidentified coliform	1	0	0	0
Gram-positive bacteria				
Arcanobacterium pyogenes	0	0	1	1
Enterococcus spp.	2	1	0	0
Streptococcus spp.	9	1	0	0

[a] Group A_1 bighorn sheep that were necropsied (n = 60) were part of a herd that was captured and maintained in a holding facility in an attempt to prevent spread of respiratory disease to other bighorn sheep in Hells Canyon metapopulation; Group A_2 bighorn sheep that were necropsied (n = 12) were free-ranging animals that died during the epizootic. [b] Bacteria isolated from lung (LG) or liver (LV) samples.

isolates with PM-5 and PM-6 profiles were cultured from group A_2 bighorn sheep.

REA of six *P. trehalosi* biovariant 2 isolates distinguished three REA profiles: PT2-1 from the group A_1 bighorn sheep; PT2-2 from four group A_2 bighorn sheep; and PT 2-3 from one group A_2 bighorn sheep, which had a REA profile identical to that of an isolate previously associated with disease in bighorn sheep, in Central Idaho.[30] The 10 *P. trehalosi* biovariant 2[B] isolates were differentiated by seven distinct REA profiles, one of which (PT-1) was cultured from both groups of bighorn sheep. Isolates with the other six profiles were cultured from either group A_1 or group A_2 bighorn sheep.

Isolation of additional etiologic agents

Mycoplasma ovipneumoniae was isolated from five of 70 OP swab samples collected from group A_1 bighorn sheep at the time of capture. All other samples were culture-negative for *Mycoplasma* spp.

Viruses were not isolated from any of the swab samples from either group A_1 or group A_2; however, one of nine cultures of lung tissue from group A_1 animals was positive for PI-3. All samples tested were negative for respiratory syncytial virus (RSV) by the immunoassay test procedure.

Serologic test results

Serologic tests conducted on 21 paired serum samples from group A_1 bighorn sheep detected antibodies against BRS, BVD, IBR, and PI-3 viruses in the majority of the samples collected at the time of capture and 9–19 days later (Table 3). Antibody titers against BVD and IBR viruses were at low levels in eight and nine samples and were undetectable in 13 and 12 paired serum samples, respectively. Antibody levels against both BRS and PI-3 viruses were detected in the first serum sampling from 19 of 21 animals at the time of capture, and ≥fourfold increases were detected against those viruses in the second sampling from three and seven animals, respectively. Titers to BRS and BVD detected in group A_2 serum samples were also comparable to those detected for group A_1 bighorn sheep; antibodies to PI-3 viruses were detected in serum samples from group A_2 but not in serum samples harvested at necropsy. Antibodies were not detected against IBR virus in serum samples from group A_2 bighorn sheep.

Pathology

Although presence of *Protostrongylus* sp. was observed in some cases, gross and microscopic observations of tissues from necropsied bighorn sheep were most consistent with bacterial pneumonia. Evaluations of tissue from animals in groups A_1 and A_2 provided comparable information and revealed that deaths were due to fibrinopurulent bronchopneumonia frequently associated with fibrinous pleuritis. Lung tissue assessed grossly at time of necropsy was severely swollen and variously discolored. Consolidation of 35–90% of total lung volume was evident in lung tissue from 51 of 60 group A_1 and from five of seven group A_2 bighorn sheep

Table 3. Prevalence of antibodies against listed viruses detected in sera from bighorn sheep with evidence of respiratory disease in the Hells Canyon metapopulation.

	Animal numbers and antibody titer range detected in sera of bighorn sheep[b]			
	Group A_1		Group A_2	
Virus[a]	At capture (n = 21)	9–19 Days postcapture (n = 21)	Captured/ released (n = 4)	Necropsied in field (n = 2)
BRS	18 (8–64)	19 (8–32)[c]	2 (8–32)	1 (32)
BVD	14 (8–32)	8 (8–16)	1 (8)	1 (8)
IBR	11 (8–32)	9 (8–16)	0	0
PI-3	15 (10–40)	19 (10–80)[c]	4 (20–80)	0
OPP	0	0	NT[d]	NT

[a] Sera were tested for antibodies against viral agents: BRS, bovine respiratory syncytial; BVD, bovine viral diarrhea; IBR, infectious bovine rhinotracheitis; PI-3, parainfluenza 3; OPP, ovine progressive pneumonia. Numbers in parentheses indicate titer ranges.

[b] Group A_1 bighorn sheep were captured and translocated to a holding facility. Group A_2 bighorn sheep consisted of free-ranging animals that were captured and released (n = 4), and bighorn sheep discovered dead or agonal and necropsied in the field (n = 2).

[c] Fourfold or greater titer increases indicative of recent viral infection were detected in sera from three bighorn sheep against BRS virus and in serum from seven bighorn sheep against PI-3 virus.

[d] NT indicates that the serum samples were not tested for antibodies against listed virus.

at necropsy. Pneumonic lesions were generally present in the cranial and caudal portions of lung and progressed dorsally toward the diaphragmatic lobes. Microscopic examination revealed necrosis with an influx of inflammatory cells, serous leakage, and overt hemorrhage. This process extended to and through the serosal lining, resulting in fibrinous clots on the surface of the lungs and reduced lung capacity. There was evidence of previous bronchopneumonia in the lungs of many animals, which most prominently affected the right cranial lung lobe, resulting in scarring and adhesion to the chest wall.

DISCUSSION

Fibrinopurulent bronchopneumonia was identified as the cause of death in both captive and free-ranging bighorn sheep and was characteristic of bacterial pneumonias in other ruminants.[19] The 1995–1996 pneumonic epizootic resulted in an estimated 50–75% mortality in affected bighorn sheep herds in Hells Canyon and nearly 90% mortality of group A_1 bighorn sheep transferred to captivity.[12] Collection of samples while the epizootic was in progress, particularly from captive bighorn sheep, provided an unprecedented and extremely valuable opportunity to conduct an investigation of the etiology associated with this epizootic. Comparison of survey results from group A_1 animals at the time of and following capture with those from free-ranging bighorn sheep were remarkably similar. Therefore, there was no evidence that the organisms isolated and pathologic observations were influenced by capture and/or antibiotic treatment.

When a specific infectious organism acts as a primary pathogen and causes widespread disease, it is generally possible to isolate that organism from all hosts or to detect specific immune responses of the hosts against that pathogen. A variety of test systems, such as those provided by the Centers for Disease Control and Prevention[13] and by others,[22] target specific genetic markers and have been developed for detecting and monitoring transmission of specific organisms associated with human diseases. However, such systems are not generally applied to diseases in animal populations. Test systems, similar to those used in human epidemiologic studies, were used in this and previous studies of organisms isolated from bighorn sheep,[30,46,49,57] and these systems provide greater reliability for assessment of disease associations in bighorn sheep.

Bacteria most commonly associated with bronchopneumonia in domestic livestock are members of the Pasteurellaceae family.[3,19,27,61] These bacteria are usually obligate parasites that commonly reside as commensals on mucous membranes of the upper respiratory and distal reproductive tracts of their hosts. The most virulent strains may act as primary pathogens in susceptible animals; however, the majority act as secondary or opportunistic pathogens in animals with immunity compromised by other factors.[55] Compromising factors may include nutritional and climatic stresses and primary infections with *Mycoplasma* spp. and/or viruses.[7,61]

The greatest amount of information with regard to *Pasteurella* spp. carried by the bighorn sheep in this study was obtained by culture of OP samples. In contrast, relatively few organisms were isolated

from NA samples. Others have also demonstrated that OP samples were superior to NA samples for culturing *Pasteurella* spp. from bighorn sheep.[58] Organisms isolated from lung samples, with the exception of *P. multocida gallicida*, were also isolated from OP samples.

The majority of bacterial isolates cultured from lung lesions of bighorn sheep that died during the Hells Canyon epizootic were identified as *P. multocida*. Capsular and somatic antigen typing and REA profile comparisons conducted in this study revealed that the organisms were very heterogeneous. Characterization of 90 additional *P. multocida* isolates associated with this epizootic further substantiated the great diversity of the isolates.[57] Results from this study demonstrate that while *P. multocida* played a major role in this epizootic, respiratory disease was not attributable to a single *P. multocida* strain.

The next most common group of *Pasteurella* isolated from lung samples were identified as *P. trehalosi*, biovariant 2[B]. However, we conclude that these organisms did not cause disease by themselves or contribute significantly to this epizootic. *Pasteurella* in this biovariant comprise one of the most common groups carried by clinically healthy bighorn sheep, as previously reported.[29] In addition, REA profiles developed with DNA from 10 of the isolates from samples collected during this epizootic did not reveal a single primary strain; rather, seven different strains were detected. Therefore, it appears that each of these organisms acted as an opportunistic pathogen, and in six of eight group A_1 and group A_2 bighorn sheep, they occurred in concert with *P. multocida* in lung tissue.

As a result of the multiplicity of *Pasteurella* species and strains isolated from the bighorn sheep, particularly lung samples of the animals, it was evident that no single strain of *Pasteurella* was the primary pathogen responsible for the epizootic. Therefore, it is critical to consider other infectious organisms that could have predisposed the bighorn sheep to disease associated with opportunistic *Pasteurella* strains, as has been demonstrated in domestic ruminants.[2,5,7,8,15,21,23,31,53,61]

Mycoplasma spp. are ubiquitous and have been isolated from practically all mammalian and avian species,[11] including North American wild sheep.[1,60] Emerging diseases associated with mycoplasmas have been reported with increasing frequency and can present a risk to wildlife species, especially during conditions of stress, such as those that occur during release from captivity into new habitats, translocation, and human encroachment into shrinking habitats.[45] Much like members of the Pasteurellaceae, many *Mycoplasma* spp. reside as commensals on the mucosa of the upper respiratory tract of asymptomatic animals.[8] They may act as opportunistic pathogens under a variety of conditions[55] and may contribute to invasion of the lungs by other bacteria by interference with tracheal ciliary action.[28,52] Pneumonia caused by *Mycoplasma* spp. in domestic sheep may initially produce minimal clinical symptoms, followed by an acute phase and then a remission phase, with persistent lung lesions and poor growth and exercise intolerance of affected lambs.[53,55] High rates of pneumonia-associated mortality in lambs have been reported in a free-ranging bighorn sheep population following a pneumonia-associated die-off.[48] The possibility of the presence of *Mycoplasma* infections in such die-offs should be considered.

Both *M. ovipneumoniae* and *Mycoplasma arginini* have been isolated from clinically healthy and pneumonic domestic sheep.[2,8] Although both species have been associated with pneumonia, *M. ovipneumoniae* is considered to be more pathogenic and commonly associated with disease.[2,4,8,44] Fatal pneumonia has been attributed to *M. ovipneumoniae* in Dall's sheep (*Ovis dalli*) following contact with domestic sheep and to *M. arginini* in bighorn sheep captured in Montana and transported to Pennsylvania.[1,6]

PI-3 viruses have been associated with respiratory disease in domestic livestock[7,21–23] and have previously been isolated from bighorn sheep in Hells Canyon,[33] Wyoming,[39] and California.[14] Isolation of PI-3 from the lung of one of nine group A_1 bighorn sheep tested and fourfold increases of antibody levels to this virus in seven of 21 group A_1 bighorn sheep provide evidence that PI-3 viruses were present in that population prior to capture and captivity. PI-3 viruses may be maintained in animal populations, and since immunity declines rapidly, reinfection is common. The viruses are capable of replicating in, and destruction of, respiratory epithelial cells as well as depressing phagocytic activity of host phagocytes, resulting in increased susceptibility of the host to secondary bacterial infections.[31] Susceptibility of domestic lambs to experimental infection with *P. haemolytica* was greatly increased by inoculation with PI-3 virus 7 days prior to challenge.[15] Our results provide evidence that PI-3 virus may have contributed to the 1995–1996 bighorn sheep epizootic.

RSVs are ubiquitous and endemic in many animal populations,[5] including bighorn sheep.[17,50] Presence of antibodies against RSV in 19 of 21 sera collected at the time of capture from Hells Canyon bighorn sheep and fourfold antibody increases in

sera of three bighorn sheep following capture indicate recent infections. However, absence of pathologic changes characteristic of RSV infections and failure to detect virus in lung tissue do not support a major role of RSV in the 1995–1996 epizootic.

Both BVD and IBR viruses are associated with respiratory disease in domestic livestock.[8,61] Low and stable antibody titers against BVD and IBR viruses were detected in some paired bighorn sheep serum samples (Table 3), although neither virus was isolated. Therefore, there was no incriminating evidence of active infections with either of these viruses in the Hells Canyon bighorn sheep epizootic.

Pneumonic epizootics in cattle and domestic sheep populations are commonly associated with multiple stress factors (e.g., shipping [translocation] and intermingling of animals carrying multiple and varied microbial flora from different populations at sale yards and feeding lots). Intermingling of domestic livestock from different herds frequently results in respiratory disease, particularly during times of stress.[21,23,61]

Between 1971 and 1995, 22 translocations of bighorn sheep were conducted to augment the Hells Canyon bighorn sheep population.[12,26] The bighorn sheep were translocated from nine different areas, including sites in Canada, Colorado, Idaho, Montana, Washington, and Wyoming. The bighorn sheep from each of those sites would have carried distinct biological packages that could have included multiple bacterial strains, viruses, and parasites with the potential to compromise the health of the translocated and/or resident bighorn sheep at the release sites.

The initial signs of disease in the Hells Canyon bighorn sheep occurred in early November, and all animals would have been subjected to stresses associated with rut. The Hells Canyon 1995–1996 bighorn sheep epizootic was associated with a complex of potentially pathogenic organisms and probable stress factors. Although it has been documented that bacteria experimentally transmitted from domestic livestock can cause fatal pneumonia in bighorn sheep,[20,37] bacteria isolated from a feral goat and two bighorn sheep found among a small group of Black Butte bighorn sheep at the initial stage of the epizootic were not detected in other bighorn sheep and did not contribute further to this epizootic. Based on the available information derived from those evaluations and the data in this report, the source of the microorganisms in this outbreak could not be determined[46,57] but could have originated from one or more sources, including resident or translocated bighorn sheep or domestic livestock in close contact.

Although treatment of captive group A_1 bighorn sheep with antibiotics did not appear to increase survival, eight of them survived in captivity for several months to years and died of causes unrelated to the epizootic. Failure of antibiotic treatment may have been due to a variety of factors including lack of efficacy, host response, and difficulty in establishing and maintaining effective levels of antibiotics to control infections caused by multiple organisms.[41] Recovery of animals from bacterial infections, whether treated or not treated with antibiotics, depends heavily on the innate abilities of their phagocytic cells to ingest and destroy bacteria and of the body to eliminate toxic by-products and to mount immune responses to infectious organisms.[41] Viral infections, nutritional deficiencies, and stress are known to suppress these abilities.[56] In addition, immunosuppression of individual animals can result in heavy shedding of infectious agents and rapid transmission to other animals.

No single bacterium or virus detected in this study was identified as the primary pathogen of the 1995–1996 Hells Canyon bighorn sheep epizootic. This could be due to possible involvement of a novel pathogen, lack of identification or isolation of a primary pathogen, or interactions of multiple organisms. Since bighorn sheep and domestic livestock carry a variety of microorganisms, there is some risk of disease introduction when animals from different populations or species are intermingled. Microbial populations residing in niches such as the surface of the oropharyngeal mucosa are dynamic populations that shift with introduction of new organisms, competition between organisms, and changes in the host's innate and acquired immunity. Although many factors may be involved in dynamic shifts, of primary importance is detection of potential pathogens within animal populations. In recognition of the complexity of the 1995–1996 Hells Canyon bighorn sheep epizootic and the multiple potential sources of etiologic agents, it is recommended that both translocated and resident bighorn sheep be tested for pathogenic organisms. Further work on interactions of multiple pathogens in the development of disease in bighorn sheep is warranted.

Acknowledgments: We notably acknowledge the important contributions of many individuals made throughout the study period. These individuals include Lloyd Oldenburg, Idaho Fish and Game; Vic Coggins, Oregon Fish and Wildlife; Pat Fowler, Washington Fish and Wildlife; Holly Akenson, for her skill and expertise in the field; Gene Majors, for his observations during the initial phase of this

die-off; the Foundation for North American Wild Sheep, for their immediate and continuing support; and Helicopter Wildlife Management, for their expert assistance during the capture of the Black Butte herd. In addition, we thank Lisa Cowan and Jeanne Bulgin for their exceptional proficiency in the Caine Center microbiology laboratory, Drs. Stuart Lincoln and Bruce Anderson for their pathology expertise and skills, and Dr. A. J. DeMassa for speciation of the *Mycoplasma* isolates.

LITERATURE CITED

1. Al-Aubaidi, J. M., W. D. Taylor, G. R. Bubash, and A. H. Dardiri. 1972. Identification and characterization of *Mycoplasma arginini* from bighorn sheep (*Ovis canadensis*) and goats. Am. J. Vet. Res. 33: 87–90.

2. Alley, M. R., J. R. Quinlan, and J. K. Clark. 1975. The prevalence of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in the respiratory tract of sheep. N. Z. Vet. J. 23: 137–141.

3. Angen, O., R. Mutters, D. A. Caugant, J. E. Olsen, and M. Bisgaard. 1999. Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov. *Mannheimia ruminalis* sp. nov. and *Mannheimia varigena* sp. nov. Int. J. Syst. Bacteriol. 49: 67–86.

4. Babu, Y. H., K. G. Murthy, and S. Krishnaswamy. 1982. Isolation and identification of *Mycoplasma* from the respiratory tract of sheep. Kerala J. Vet. Sci. 13: 79–88.

5. Baker, J. C., J. A. Ellis, and E. G. Clark. 1997. Bovine respiratory syncytial virus. *In:* St. Jean, G., and J. Vestweber (eds.). The Veterinary Clinics of North America: Food Animal Practice. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 425–454.

6. Black, S. R., I. K. Barker, K. G. Mehren, G. J. Crawshaw, S. Rosendal, L. Ruhnke, J. Thorsen, and P. S. Carman. 1988. An epizootic of *Mycoplasma ovipneumoniae* infection in captive Dall's sheep (*Ovis dalli dalli*). J. Wildl. Dis. 24: 627–635.

7. Brogden, K. A., H. D. Lehmkuhl, and R. C. Cutlip. 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Vet. Res. 29: 233–254.

8. Brogden, K. A., D. Rose, R. C. Cutlip, H. D. Lehmkuhl, and J. G. Tully. 1988. Isolation and identification of *Mycoplasmas* from the nasal cavity of sheep. Am. J. Vet. Res. 49: 1669–1672.

9. Buechner, H. K. 1960. The bighorn sheep in the United States, its past, present, and future. Wildl. Monogr. 4: 1–174.

10. Carter, G. R., and J. R. Cole. (eds.). 1990. Diagnostic Procedures in Veterinary Bacteriology and Mycology, 5th ed. *Pasteurella* and *Francisella*. Academic Press, New York, New York. Pp. 129–142.

11. Cassel, G. H., W. A. Clyde, and J. K. Davis. 1985. Mycoplasma respiratory infections. *In:* Razin, S., and M. F. Barile (eds.). The Mycoplasmas, vol. 4. Mycoplasma Pathogenicity. Academic Press, New York, New York. Pp. 65–106.

12. Cassirer, E. F., L. E. Oldenburg, V. L. Coggins, P. Fowler, K. Rudolph, D. L. Hunter, and W. J. Foreyt. 1996. Overview and preliminary analysis of a bighorn sheep die-off, Hells Canyon 1995–96. Bienn. Symp. North. Wild Sheep Goat Counc. 10: 78–86.

13. Centers for Disease Control and Prevention. 1996. Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis: A Manual. National Center for Infectious Diseases, Atlanta, Georgia.

14. Clark, R. K., D. A. Jessup, M. D. Kock, and R. A. Weaver. 1985. Survey of desert bighorn sheep in California for exposure of selected infectious diseases. J. Am. Vet. Med. Assoc. 187: 1175–1179.

15. Davies, D. H., M. Herceg, B. A. H. Jones, and D. C. Thurley. 1981. The pathogenesis of sequential infection with parainfluenza virus type 3 and *Pasteurella haemolytica* in sheep. Vet. Microbiol. 6: 173–182.

16. DeForge, J. R., D. A. Jessup, C. W. Jenner, and J. E. Scott. 1982. Disease investigations into high lamb mortality of desert bighorn in the Santa Rosa Mountains, California. Desert Bighorn Counc. 1982 Trans. 26: 76–81.

17. Dunbar, M. R., D. A. Jessup, J. F. Evermann, and W. J. Foreyt. 1985. Seroprevalence of respiratory syncytial virus in free-ranging bighorn sheep. J. Am. Vet. Med. Assoc. 187: 1173–1174.

18. Dunbar, M. R., A. C. S. Ward, and G. Power. 1990. Isolation of *Pasteurella haemolytica* from tonsillar biopsies of Rocky Mountain bighorn sheep. J. Wildl. Dis. 26: 210–213.

19. Dungworth, D. L. 1993. The respiratory system. *In:* Jubb, K. V. F., P. C. Kennedy, and N. Palmer (eds.). Pathology of Domestic Animals, 4th ed., vol. 2. Academic Press, New York, New York. Pp. 539–699.

20. Foreyt, W. J., K. P. Snipes, and R. W. Kasten. 1994. Fatal pneumonia following inoculation of healthy bighorn sheep with *Pasteurella haemolytica* from healthy domestic sheep. J. Wildl. Dis. 30: 137–145.

21. Frank, G. H. 1989. Pasteurellosis of cattle. *In:* Adlam, C., and J. M. Rutter (eds.). *Pasteurella* and Pasteurellosis. Academic Press, London, U.K. Pp. 223–262.

22. Gautom, R. K. 1997. Rapid pulsed-field electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Microbiol. 35: 2977–2980.

23. Gilmour, N. J. L., and J. S. Gilmour. 1989. Pasteurellosis of sheep. *In:* Adlam, C., and J. M. Rutter (eds.). *Pasteurella* and Pasteurellosis. Academic Press, London, U.K. Pp. 223–262.

24. Goll, F. 1994. Identification of mycoplasmas isolated from domestic animals. *In:* Whitford, H. W., R. F. Rosenbusch, and L. H. Lauerman (eds.). Mycoplasmosis in Animals: Laboratory Diagnosis. Iowa State Univ. Press, Ames, Iowa. Pp. 15–30.

25. Hayflick, L. 1965. Tissue cultures and mycoplasmas. Tex. Rep. Biol. Med. 23: 285–303.

26. Hells Canyon Bighorn Sheep Restoration Committee. 2004. The Hells Canyon Initiative Hells Canyon Big-

horn Sheep Restoration Plan. Idaho Department of Fish and Game, Lewiston, Idaho. Pp. 39.

27. Hodgson, J. C., G. M. Moon, M. Quirie, and W. Donachie. 2003. Association of LPS chemotype of *Mannheimia* (*Pasteurella*) *haemolytica* A1 with disease virulence in a model of ovine pneumonic pasteurellosis. J. Endotoxin Res. 9: 25–32.

28. Howard, C. J., and L. H. Thomas. 1974. Inhibition of *Mycoplasma dispar* of ciliary activity in tracheal organ cultures. Infect. Immun. 10: 405–408.

29. Jaworski, M. D., D. L. Hunter, and A. C. S. Ward. 1998. Biovariants of isolates of *Pasteurella* from domestic and wild ruminants. J. Vet. Diagn. Invest. 10: 49–55.

30. Jaworski, M. D., A. C. S. Ward, D. L. Hunter, and I. V. Wesley. 1993. Use of DNA analysis of *Pasteurella haemolytica* biotype T isolates to monitor transmission in bighorn sheep (*Ovis canadensis canadensis*). J. Clin. Microbiol. 31: 831–835.

31. Kahrs, R. F. 2001. Parainfluenza type-3. *In:* Viral Disease of Cattle. Iowa State Univ. Press, Ames, Iowa. Pp. 185–193.

32. McCarty, C. W., and M. W. Miller. 1998. Modeling the Population Dynamics of Bighorn Sheep: A Synthesis of Literature. Colorado Division of Wildlife Special Report No. 73. Colorado Division of Wildlife, Ft. Collins, Colorado. 35 pp.

33. McNeill, G. R., B. Rybarczyk, and D. Anderson. 1987. Progress reports, surveys and inventory. *In:* Oldenburg, L. E., and R. A. Meyer (eds.). Job Progress Reports, Study I Job 4, Bighorn Sheep. Statewide Surveys and Inventory, July 1, 1986 to June 30, 1987. Project W-170-R-11. Idaho Department of Fish and Game, Boise, Idaho. Pp. 1–10.

34. Miller, M. W. 2001. Pasteurellosis. *In:* Williams, E. S., and I. K. Barker (eds.). Infectious Diseases of Wild Mammals, 3rd ed. Iowa State Univ. Press, Ames, Iowa. Pp. 330–339.

35. Miller, M. W., N. T. Hobbs, and E. S. Williams. 1991. Spontaneous pasteurellosis in captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): clinical, laboratory, and epizootiological observations. J. Wildl. Dis. 27: 534–542.

36. Miller, M. W., J. E. Vayhinger, D. C. Bowden, S. Roush, T. Verry, A. N. Torres, and V. D. Jurgens. 2000. Drug treatment for lungworm in bighorn sheep: reevaluation of a 20-year-old management prescription. J. Wildl. Manag. 64: 505–512.

37. 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*. Can. J. Vet. Res. 52: 439–444.

38. Onderka, D. K., and W. D. Wishart. 1984. A major bighorn sheep die-off from pneumonia in Southern Alberta. Bienn. Symp. North. Wild. Sheep Goat Counc. 4: 356–363.

39. Parks, J. B., G. Post, T. Thorne, and P. Nash. 1972. Parainfluenza-3 virus infection in Rocky Mountain bighorn sheep. J. Am. Vet. Med. Assoc. 161: 670–672.

40. Post, G. 1962. Pasteurellosis of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). J. Wildl. Dis. 23: 1–14.

41. Prescott, J. F., and J. D. Baggot (eds.). 1993. Antimicrobial therapy in veterinary medicine, 2nd ed. Iowa State Univ. Press, Ames, Iowa. Pp. 11–60.

42. Queen, C., A. C. S. Ward, and D. L. Hunter. 1994. Bacteria isolated from nasal and tonsillar samples of clinically healthy Rocky Mountain bighorn and domestic sheep. J. Wildl. Dis. 30: 1–7.

43. Rimler, R. B., and K. A. Brogden. 1986. *Pasteurella multocida* isolated from rabbits and swine. Am. J. Vet. Res. 47: 730–737.

44. Rosendal, S. 1994. Ovine and caprine mycoplasmas. *In:* Whitford, W. H., R. F. Rosenbusch, and L. H. Lauerman (eds.). Mycoplasmosis in Animals: Laboratory Diagnosis. Iowa State Univ. Press, Ames, Iowa. Pp. 84–96.

45. Rosengarten, R., C. Citti, P. Much, J. Spergser, M. Droesse, and M. Hewicker-Trautwein. 2001. The changing image of *Mycoplasmas*: from innocent bystanders to emerging and reemerging pathogens in human and animal diseases. *In:* Muhldorfer, I., and S. K. Schafer (eds.). Emerging Bacterial Pathogens, vol. 8. Karger Publishing, Basel, Switzerland. Pp. 166–185.

46. Rudolph, K. M., D. L. Hunter, W. J. Foreyt, E. F. Cassirer, R. B. Rimler, and A. C. S. Ward. 2003. Sharing of *Pasteurella* spp. between free-ranging bighorn sheep and feral goats. J. Wildl. Dis. 39: 897–903.

47. Ryder, T. J., E. S. Williams, and S. L. Anderson. 1994. Residual effects of pneumonia in the bighorn sheep of Whiskey Mountain Wyoming. Bienn. Symp. North. Wild Sheep Goat Counc. 9: 15–19.

48. Ryder, T. J., E. S. Williams, K. W. Mills, K. H. Bowels, and E. T. Thorne. 1992. Effects of pneumonia on population size and lamb recruitment in Whiskey Mountain bighorn sheep. Bienn. Symp. North. Wild Sheep Goat Counc. 8: 136–146.

49. Snipes, K. P., R. W. Kasten, M. A. Wild, M. W. Miller, D. A. Jessup, R. L. Silflow, W. J. Foreyt, and T. E. Carpenter. 1992. Using ribosomal RNA gene restriction patterns in distinguishing isolates of *Pasteurella haemolytica* from bighorn sheep (*Ovis canadensis*). J. Wildl. Dis. 28: 347–354.

50. Spraker, T. R., and J. K. Collins. 1985. Isolation and serologic evidence of a respiratory syncytial virus in bighorn sheep from Colorado. J. Wildl. Dis. 22: 416–418.

51. Spraker, T. R., and C. P. Hibler. 1982. An overview of the clinical signs, gross and histological lesions of the pneumonia complex of bighorn sheep. Bienn. Symp. North. Wild Sheep Goat Counc. 3: 163–172.

52. St. George, T. D., and L. E. Carmichael. 1975. Isolation of *Mycoplasma ovipneumoniae* from sheep with chronic pneumonia. Vet. Rec. 97: 205–206.

53. Sullivan, N. D., T. D. St. George, and N. Horsfall. 1973. A proliferative interstitial pneumonia of sheep associated with mycoplasma infection: 1. Natural history of the disease in a flock. Austral Vet. J. 49: 57–62.

54. Taylor, R. E. L. 1976. Mortality of Nevada bighorn sheep from pneumonia. Desert Bighorn Counc. Trans. 20: 51–52.

55. Timoney, J. F., J. H. Gillespie, F. W. Scott, and J. E. Barlough (eds.). 1988. The genera *Mycoplasma* and *Ureaplasma*. *In:* Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th ed. Comstock Publishing, Ithaca, New York. Pp. 295–317.

56. Tizard, I. R. (ed.). 1996. Veterinary Immunology: An Introduction. W. B. Saunders Co., Philadelphia, Pennsylvania. 493 pp.

57. Weiser, G. C., W. J. DeLong, J. L. Paz, W. J. Price, B. Shafii, and A. C. S. Ward. 2003. Characterization of *Pasteurella multocida* associated with pneumonia in bighorn sheep. J. Wildl. Dis. 39: 536–544.

58. Wild, M. A., and M. W. Miller. 1991. Detecting nonhemolytic *Pasteurella haemolytica* infections in healthy Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): influence of sample site and handling. J. Wildl. Dis. 27: 53–60.

59. Wilson, M. A., R. B. Rimler, and L. J. Hoffman. 1992. Comparison of DNA fingerprints and somatic serotypes of serogroup B and E *Pasteurella multocida* isolates. J. Clin. Microbiol. 30: 1518–1524.

60. Woolf, A., D. C. Kradel, and G. R. Babash. 1970. *Mycoplasma* isolates from pneumonia in captive Rocky Mountain bighorn sheep. J. Wildl. Dis. 6: 169–170.

61. Yates, W. D. G. 1982. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. Can. J. Comp. Med. 46: 225–263.

Received for publication 6 October 2006