

SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 48(1), 2012, pp. 168–172
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Survival of Bighorn Sheep (*Ovis canadensis*) Commingled with Domestic Sheep (*Ovis aries*) in the Absence of *Mycoplasma ovipneumoniae*

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ABSTRACT: To test the hypothesis that *Mycoplasma ovipneumoniae* is an important agent of the bighorn sheep (*Ovis canadensis*) pneumonia that has previously inevitably followed experimental commingling with domestic sheep (*Ovis aries*), we commingled *M. ovipneumoniae*-free domestic and bighorn sheep ($n=4$ each). One bighorn sheep died with acute pneumonia 90 days after commingling, but the other three remained healthy for >100 days. This unprecedented survival rate is significantly different ($P=0.002$) from that of previous bighorn-domestic sheep contact studies but similar to ($P>0.05$) bighorn sheep survival following commingling with other ungulates. The absence of epizootic respiratory disease in this experiment supports the hypothesized role of *M. ovipneumoniae* as a key pathogen of epizootic pneumonia in bighorn sheep commingled with domestic sheep.

Key words: Bighorn sheep, domestic sheep, *Mycoplasma ovipneumoniae*, pneumonia.

Recovery of bighorn sheep (*Ovis canadensis*) populations in western North America has been hindered by disease, particularly pneumonia (e.g., Miller, 2001). Over the past 100 yr, acute pneumonia outbreaks in bighorn sheep have been reported to frequently follow contacts with domestic Caprinae, particularly sheep (*Ovis aries*), and to relatively rarely follow contacts with other domestic or wild animals (Grinnell, 1928; Buechner, 1960; Goodson, 1982; George et al., 2008; Wolfe et al., 2010). Similarly, experimental commingling of bighorn sheep with domestic sheep or mouflon (*O. aries orientalis*) has

consistently resulted in bighorn sheep mortality approaching 100% within 90 days, whereas commingling with other domestic livestock, including cattle (*Bos taurus*), llamas (*Lama glama*), horses (*Equus caballus*), and goats (*Capra hircus*), has resulted in <30% bighorn sheep mortality, and commingling with native ungulates, including deer (*Odocoileus hemionus* and *Odocoileus virginianus*), elk (*Cervus elaphus*), and mountain goats (*Oreamnos americanus*), has resulted in no observed bighorn sheep mortality (Table 1).

Mycoplasma ovipneumoniae has been implicated in pneumonia in free-ranging bighorn sheep (Rudolph et al., 2007; Besser et al., 2008; Wolfe et al., 2010) and in bighorn sheep pneumonia following experimental contact with domestic sheep (Lawrence et al., 2010). Evidence of *M. ovipneumoniae* involvement was also detected in archived materials available from three earlier bighorn sheep-domestic sheep contact studies, including specific 16S rDNA sequences in formalin-fixed, paraffin-embedded affected lung tissues (Foreyt, 1989, 1994) and *M. ovipneumoniae* seroconversion in a rare bighorn sheep that survived contact (Foreyt, 1992b; data not shown). Our objective was to determine the outcome of contact of bighorn sheep with domestic sheep in the absence of *M. ovipneumoniae*.

This study was carried out in accordance with United States Department of

TABLE 1. Survival of bighorn sheep (BHS; *Ovis canadensis*) experimentally commingled with other domestic or wild ungulates in previous studies.

Contact species (n) ^a	BHS (n) ^b	Mortality (%)	Contact day (n BHS died)	Space (ha)	Reference
<i>Ovis</i> sp.					
Dom. sheep (11)	14	93	26 (7), 72 (6)	2.5	Foreyt and Jessup, 1982
Dom. sheep (>100)	37	100	25 (8), 31 (5), 63 (24)	445	Foreyt and Jessup, 1982
Dom. sheep (2)	2	100	29 (2)	0.004	Onderka and Wishart, 1988
Dom. sheep (6)	6	100	4 (1), 27 (2), 29 (1), 36 (1), 71 (1)	2	Foreyt, 1989
Dom. sheep (2)	2	100	14 (2)	0.006	Foreyt, 1990
Dom. sheep/mouflon (30)	5	100	11 (1), 14 (1), 17 (1), 30 (1), 99 (1)	0.27	Callan et al., 1991
Dom. sheep (4)	6	83	26 (1), 33 (1), 34 (1), 35 (1), 40 (1)	2.5	Foreyt, 1992b
Mouflon (5)	6	100	41 (2), 42 (4)	0.4	Foreyt, 1994
Dom. sheep (2)	2	100	6 (1), 8 (1)	0.002	Foreyt, 1994
Dom. sheep (3)	6	100	20 (1), 30 (4), 32 (1), 61 (1)	0.6	Foreyt, 1998
Dom. sheep (4)	4	100	67 (1), 70 (2), 74 (1)	0.02	Lawrence et al., 2010
Total	90	98			
Other Dom. spp.					
Dom. goats (3)	3	0	NA ^c	0.4	Foreyt, 1994
Llamas (3)	9	0	NA	0.8	Foreyt, 1994
Cattle (3)	4	0	NA	0.4	Foreyt, 1994
Horses (3)	6	17	22 (1)	0.5	Foreyt and Lagerquist, 1996
Cattle (3)	5	20	6 (1)	0.5	Foreyt and Lagerquist, 1996
Dom. goats (4)	7	29	“Third month” (2)	0.6	Foreyt et al., 2009
Total	34	12			
Wild ungulates					
Mt. goats (2)	9	0	NA	0.8	Foreyt, 1994
Elk (4), deer (3)	10	0	NA	0.72	Foreyt, 1992a
Elk (4)	3	0	NA	0.4	Foreyt, 1992a
Total	22	0			

^a Contact species = animal species placed in contact with bighorn sheep in each experiment. n = No. of the contact species animals in each experiment. Dom. sheep (*Ovis aries*); mouflon (*O. aries orientalis*); Dom. goats (*Capra hircus*), llamas (*Lama glama*); cattle (*Bos taurus*); horses (*Equus caballus*); Mt. goats (*Oreamnos americanus*); elk (*Cervus elaphus*); deer (*Odocoileus hemionus* and *Odocoileus virginianus*). Dom. = domestic; Mt. = mountain.

^b No. of bighorn sheep included in each contact experiment.

^c NA = Not applicable.

Agriculture animal research guidelines, under a protocol approved by the Washington State University Institutional Animal Care and Use Committee, between 15 June and 19 October 2009. Four hand-reared captive bighorn sheep, including three yearling rams (Nos. 82, 86, and 89) and one yearling ewe (07) from the Washington State University herd, and four domestic sheep, including three adult ewes (00, 01, 02) and one ewe lamb (04) purchased from a private producer in Whitman County, Washington, were used in this experiment. Prior to commingling, neither herd exhibited evidence of respiratory disease. Both herds were confirmed

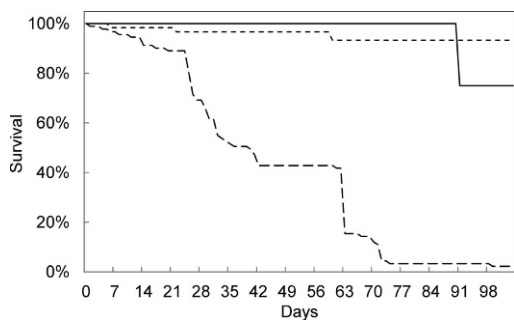


FIGURE 1. Survival of bighorn sheep (*Ovis canadensis*) commingled with *Mycoplasma ovipneumoniae*-free domestic sheep (*Ovis aries*). Kaplan-Meier survival curve plots of bighorn sheep placed in contact with domestic sheep in 11 previous experiments ($n=90$, ----), bighorn sheep placed in contact with other ungulates in nine previous experiments ($n=56$, ---), and bighorn sheep placed in contact with *M. ovipneumoniae*-free domestic sheep in this study ($n=4$, ———).

to be uniformly negative by serology, culture, and polymerase chain reaction tests for *M. ovipneumoniae* conducted using the routine methods of the Washington Animal Disease Diagnostic Laboratory, a laboratory fully accredited by the American Association of Veterinary Laboratory Diagnosticians (validation data and SOPs are available from http://www.vetmed.wsu.edu/depts_waddl/).

The eight animals were commingled in a 232-m² enclosure beginning 7 July 2009. Observation for adverse health effects, particularly respiratory disease, was conducted daily by animal care staff and approximately 6 days each week by research staff. No symptoms of respiratory disease were detected in the commingled animals until 90 days after commingling, when a single bighorn ram (86) developed clinical signs of pneumonia and died <12 hr later. The remaining three bighorn sheep exhibited no signs of respiratory disease through the termination of the experiment on day 104. Bighorn sheep survival in this study was compared to survival in previous bighorn sheep-domestic sheep/mouflon commingling studies (2% survival) and to survival in previous bighorn sheep-other ungulate

commingling studies (93% survival) using Kaplan-Meier survival analysis and the log-rank test (R, version 2.11.1, <http://www.r-project.org/>). Bighorn sheep survival in this experiment (75% for 104 days) was significantly higher than that in previous bighorn-domestic sheep commingling experiments (log-rank test, $P<0.002$) but did not differ significantly from bighorn sheep survival after experimental commingling with nonsheep ungulates (Fig. 1).

Necropsy of bighorn sheep 86 identified acute bronchopneumonia as the cause of death, with congestion, hemorrhage, necrotic neutrophils (oat cells), and numerous bacterial colonies seen on histopathologic evaluation. Fibrin and neutrophils were observed on the pleura and severe fibrin deposition and edema was observed in pulmonary interlobular septa. Conventional aerobic bacterial cultures of lung lesions, tracheobronchial lymph nodes, and nasal and pharyngeal swabs identified *Manheimia haemolytica* in high numbers, accompanied by moderate numbers of *Bibersteinia trehalosi*, at all respiratory sites tested, and these identifications were confirmed by species-specific PCR (Dassanayake et al., 2010). *Mycoplasma* cultures and *M. ovipneumoniae*-specific PCR of lung, tracheobronchial lymph node, middle ear, pharynx, and nose were negative. The surviving animals remained antibody negative to *M. ovipneumoniae*, ovine progressive pneumonia virus, and respiratory syncytial virus. The animals were antibody positive to PI-3 virus, but no significant titer changes were observed.

The significant finding of this study was the unprecedented majority survival of bighorn sheep commingled with domestic sheep in the absence of *M. ovipneumoniae*. This finding is consistent with the hypothesis that *M. ovipneumoniae* is an important agent in epidemic pneumonia in bighorn sheep, but additional research will be required to further substantiate this hypothesis. If this hypothesis is correct, it is possible that the risk of bighorn sheep pneumonia following contact with domestic

sheep could be significantly reduced if domestic sheep that may come into contact with bighorn sheep could be managed to eliminate carriage and shedding of *M. ovipneumoniae*.

This work was funded in part by the Wyoming Wildlife/Livestock Disease Partnership, the Idaho Department of Fish and Game, the Washington State Department of Fish and Wildlife, Nevada Bighorns Unlimited Reno, the Oregon Chapter of the Wild Sheep Foundation, the University of Idaho through the Idaho Wildlife/Domestic Animal Disease Research Oversight Committee, the Animal Disease Research Unit of the USDA Agricultural Research Service based in Pullman, Washington, and Federal Aid to Wildlife Restoration Project W-160-R administered through the Idaho Department of Fish and Game. Animal studies were supported by facilities and personnel of the Animal Disease Research Unit. These funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We sincerely thank Duane Chandler and Amy Hetrick for assistance with animal care, handling, and restraint or sample collection; George Barrington for providing access to *M. ovipneumoniae*-free sheep; Katie Baker, Stephanie Wright, Charlene Teitzel, and Dena Mellick for skilled technical assistance; Maggie Highland for detailed editorial suggestions; and Shannon Lee Swist for her role as the principal investigator of the Wyoming Wildlife/Livestock Disease Partnership funding.

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Submitted for publication 6 April 2011.

Accepted 11 August 2011.