AN ECOLOGIC STUDY COMPARING DISTRIBUTION OF PASTEURELLA TREHALOSI AND MANNHEIMIA HAEMOLYTICA BETWEEN SIERRA NEVADA BIGHORN SHEEP, WHITE MOUNTAIN BIGHORN SHEEP, AND DOMESTIC SHEEP

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Source: Journal of Wildlife Diseases, 45(4) : 930-940

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.4.930

Journal of Wildlife Diseases, 45(4), 2009, pp. 930–940

AN ECOLOGIC STUDY COMPARING DISTRIBUTION OF *PASTEURELLA TREHALOSI* AND *MANNHEIMIA HAEMOLYTICA* BETWEEN SIERRA NEVADA BIGHORN SHEEP, WHITE MOUNTAIN BIGHORN SHEEP, AND DOMESTIC SHEEP

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ABSTRACT: The prevalence and phenotypic variability of *Pasteurella* and *Mannheimia* isolates from Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*), White Mountain bighorn sheep (*Ovis canadensis nelsoni*), and domestic sheep (*Ovis aries*) from California, USA, were compared. The White Mountain bighorn sheep population had a recent history of pneumonia-associated mortality, whereas the Sierra Nevada bighorn sheep population had no recent history of pneumonia-associated mortality. The domestic sheep flocks were pastured in areas geographically near both populations but were not known to have direct contact with either bighorn sheep population. Oropharyngeal swab samples were collected from healthy domestic and bighorn sheep and cultured to characterize bacterial species, hemolysis, biogroups, and biovariants. *Pasteurella trehalosi* and *Mannheimia haemolytica* were detected in all of the study populations, but the relative proportion of each bacterial species differed among sheep populations. *Pasteurella trehalosi* was more common than *M. haemolytica* in the bighorn sheep populations, whereas the opposite was true in domestic sheep. *Mannheimia haemolytica* was separated into 11 biogroups, and *P. trehalosi* was characterized into two biogroups. Biogroup distributions for *M. haemolytica* and *P. trehalosi* differed among the three populations; however, no difference was detected for the distribution of *P. trehalosi* biogroups between the Sierra Nevada bighorn sheep and domestic sheep. The prevalence odds ratios (pOR) for the distribution of *M. haemolytica* biogroups suggested little difference between White Mountain bighorn sheep and domestic sheep compared with Sierra Nevada bighorn sheep and domestic sheep, although these comparisons had relatively large confidence intervals for the point estimates. Hemolytic activity of the isolates was not different among the sheep populations for *M. haemolytica* but was different for *P. trehalosi*. No clear evidence of association was found in the Pasteurellaceae distribution between the White Mountains bighorn sheep and domestic sheep.

Key words: Bighorn sheep, domestic sheep, ecologic study, *Mannheimia haemolytica*, *Pasteurella trehalosi*.

INTRODUCTION

During the past two centuries, the number of native bighorn sheep in North America has declined drastically (Valdez and Krausman, 1999). Native populations of Rocky Mountain (*Ovis canadensis canadensis*) and California bighorn sheep (*Ovis canadensis californiana*) have disappeared from the states of Washington, Oregon, New Mexico, USA, and regions of southwestern Idaho, northeastern California, and northwestern Nevada, USA (Shackleton et al., 1999). In addition, native desert bighorn sheep (*Ovis canadensis mexicana*) have disappeared in Texas, USA, and the Mexican states of Chihuahua and Coahuila (Krausman et al., 1999).

The major decline of bighorn sheep populations occurred during the latter half of the 19th and the early part of the 20th centuries (Valdez and Krausman, 1999). This decline corresponded to a period that cattle and domestic sheep overgrazed much of the western United States and southwestern Canada. This was especially true with regard to the population decline of Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*), which began in the mid-1800s, following the immigration of Europeans to the Sierra Nevada territory, and continued through most of the 20th

century. As a consequence, this unique taxon is now listed as endangered under the California Endangered Species Act and the Federal Endangered Species Act (Valdez and Krausman, 1999; US Fish and Wildlife Service, 2003). Although the population decline of the Sierra Nevada bighorn sheep population was well documented, the actual cause was not identified.

There is circumstantial evidence that the presence of domestic sheep in the bighorn sheep habitat has had a direct impact on bighorn sheep populations. Documented bighorn sheep die-offs in the western United States where cohabitation occurred were noted as early as the mid-1800s (Goodson, 1982). Furthermore, bighorn sheep populations failed to increase on habitats that were shared with domestic sheep (Goodson, 1982). In one unplanned experiment, accidental contact with domestic sheep intensively grazing beside a bighorn sheep enclosure was associated with all of the bighorn sheep in the enclosure dying (Foreyt and Jessup, 1982). Although the concern for the health of bighorn sheep populations contacting domestic sheep was justified by field observations, the specific reasons for the bighorn sheep die-offs are not known, although the spread of infectious diseases among the populations was suspected.

From the mid-1800s until the 1980s, pneumonia was frequently diagnosed in diseased bighorn found in the field, and bacteria from the Pasteurellaceae family were isolated several times from these animals (Martin et al., 1996). One of the challenges in summarizing the literature describing pneumonic disease in the bighorn sheep is the constantly evolving nomenclature describing the primary suspected pathogen, *Pasteurella haemolytica*. Specifically, in the past 20 yr, the nomenclature describing *P. haemolytica* has undergone several revisions and resulted in splitting and renaming of the members of this former species. The T biotypes, able to ferment trehalose, were reclassified in 1990 as *Pasteurella trehalosi* and as *Bibersteinia trehalosi* in 2007 (Blackall et al., 2007). The A biotypes, able to ferment arabinose, were reclassified in 1999 as *Mannheimia haemolytica*. Both of these species still belong to the Pasteurellaceae family (Boyce et al., 2004). In this article, we will refer to *Pasteurella trehalosi* according to the nomenclature before 2007 and to *Mannheimia haemolytica* according to the nomenclature after 1999.

The hypothesis derived from field observations was that *M. haemolytica* was the major pathogen moving from domestic sheep populations to bighorn sheep populations. This hypothesis is difficult to test in the field, where direct pathogen transmission is difficult to substantiate. In response to this, controlled contact and pathogen-challenge studies were conducted during the 1980s and 1990s.

In two experimental trials, healthy domestic sheep carrying Pasteurellaceae bacteria were introduced into pastures holding bighorn sheep; from which, all cultures for these isolates were negative. Following the introduction of the domestic animals, all of the wild sheep died from pneumonia, and *M. haemolytica* and *P. trehalosi* were isolated and presumed to be the etiologic agents (Onderka and Wishart, 1988; Foreyt, 1989; Martin et al., 1996). In another experiment, captive bighorn sheep were inoculated with *M. haemolytica* cultured from the respiratory tracts of healthy domestic sheep. The study revealed that a specific isolate of *M. haemolytica* obtained from healthy domestic sheep was lethal in bighorn sheep, but domestic sheep that were inoculated with the same dose and isolate of the bacterium did not develop respiratory disease (Foreyt et al., 1994).

A likely explanation for the differential susceptibility to infections with the same organism between bighorn and domestic sheep is the host response to the bacteria and in particular to its secreted toxins. An important virulence factor contributing to the pathogenesis of pneumonic pasteurel-

losis is leukotoxin (LKT). It is a soluble toxin, produced by *M. haemolytica* and *P. trehalosi* (Fisher et al., 1999). Neutrophils derived from bighorn sheep appear to be more susceptible to the toxin than those from domestic sheep (Silflow et al., 1993; Silflow and Foreyt, 1994; Sweeney et al., 1994). It has also been shown that *Pasteurella* and *Mannheimia* isolates exhibiting β-hemolysis on sheep blood agar usually carry the structural leukotoxin gene, *lktA* (Fisher et al., 1999). Moreover, most of these isolates present in domestic animals, as either commensal or pathogens, produce β-hemolysis. In contrast, isolates from wild ruminants are usually nonhemolytic, and the β-hemolytic isolates are more likely to cause disease in these animals (Onderka et al., 1988).

It is recognized that the Pasteurellaceae bacteria can be further differentiated using either variation in substrata metabolism (biogroups and biovariants) or serologic differentiation (serotypes). Both these methods differentiate the species into numerous categories; however, many isolates cannot be serotyped because of cross-agglutination reactions or nonreactivity in existing typing sera (Jaworski et al., 1993; Angen et al., 1999). In contrast, differentiation of biogroups and biovariants based on biochemical heterogeneity, generally works on all isolates and, for this reason, is useful in epidemiologic studies (Jaworski et al., 1998).

The objective of our study was to investigate the distribution of *P. trehalosi* and *M. haemolytica* among two populations of bighorn sheep (one with, and one without, a recent history of pneumonia-associated mortality) and a domestic sheep population that grazed within the general region of these bighorn sheep populations. The study hypothesis was (using field collected data) that a bighorn sheep population with recent history of pneumonia would have a distribution of *P. trehalosi* and *M. haemolytica* strains similar to those recovered from domestic sheep compared with a population of bighorn sheep without a recent history of pneumonia.

MATERIALS AND METHODS

Study populations

Animals from three sheep populations, Sierra Nevada bighorn sheep (SNBS), White Mountain bighorn sheep (WMBS), and domestic sheep (DS), were included in our study. The design was an ecologic study (Morgenstern, 1998), in which healthy animals were sampled from populations with different disease and spatial attributes. Specifically, the SNBS population, although designated as an endangered population, has not experienced pneumonia-associated mortality in recent years and has fewer opportunities for contact with grazing domestic sheep. The WMBS population is not designated as an endangered population but has recently exhibited pneumonia-associated mortality, consisting primarily of lamb loss. The likelihood of contact between DS and WMBS is unknown. The band of DS sampled for this study came from central eastern California, USA, and is taken as representative of domestic sheep grazing in pastures spatially related to bighorn sheep populations, but no contact between this flock of DS and the two wild study populations is known to have occurred.

Study area

The three populations were from discrete geographic sites in California, USA. The geographic range of the SNBS is patchy, primarily on the eastern edge of the Sierra Nevada mountains in California, USA (36°14′N to 38°17′N, 118°20′W to 119°33′W), and includes portions of the Inyo (36°14′N to 38°01′N, 118°01′W to 119°20′W) and Humboldt-Toiyabe National Forests (38°01′N to 38°16′N, 119°13′W to 119°31′W), Yosemite (37°45′N to 38°08′N, 119°11′W to 119°29′W) and Sequoia-Kings Canyon National Parks (36°20′N to 37°16′N, 118°21′W to 118°49′W), and other public lands in Inyo, Mono, Tuolumne, Mariposa, Madera, Fresno, and Tulare counties, USA. For this study, animals from the SNBS population were captured and sampled from three management units (northern, central, and southern) in Inyo and Mono counties, USA; each section including one or several locations (Table 1).

The range of the WMBS extends throughout the entirety of the White Mountain chain (37°26′N to 37°52′N, 118°05′W to 118°20′W). Within their range, the animals were captured and sampled from five distinct locations in Inyo and Mono counties, USA (Table 1). Domestic sheep graze on 17 US Forest Service allotments on land between the Sierra Nevada

TABLE 1. Locations in Inyo and Mono counties, California, USA, where bighorn and domestic sheep were captured and sampled between 2001 and 2006.[a]

SNBS				
Northern	Central	Southern	WMBS	DS
Mt. Warren	Wheeler Ridge	Sawmill Canyon	Silver Canyon	Sherwin Creek
Lundy Canyon		Mt. Baxter	Jeffrey Canyon	Deadman Creek
Mt. Gibbs		Mt. Langley	Willow Creek	Rock Creek
Deer Creek		Goodale Creek	Birch Creek	
Lee Vining Canyon			Rock Creek	
Mt. Wood				

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

Mountains and the White Mountains (37°30′N to 38°17′N, 118°30′W to 119°33′W). For our study, animals from two flocks of DS were captured and sampled. These flocks were located in three locations of the Inyo National Forest in Mono County, USA (Table 1).

Capture and sampling methods

Between 2001 and 2006, animals from the three populations were captured and sampled. The bighorn sheep were captured using either a net gun fired from a helicopter or a drive net with the aid of a helicopter. Animals captured by net gun were located by ground surveillance and chase times were limited to ≤5 min. All captured animals were physically restrained, hobbled, blindfolded, and transported to a base camp, where oropharyngeal swab samples were collected. All animals were subsequently returned to their original capture site after processing. The data from this study came from apparently healthy animals only. The DS were captured and restrained by the owner's employees, and members of the research team collected oropharyngeal swab samples.

Following sampling, the oropharyngeal swabs were placed in a Port-a-cul® tube (Fisher Scientific, Houston, Texas, USA), containing modified Cary-Blair media, and stored for transport in an insulated cooled container. Within 24–36 hr, the stored oropharyngeal swabs were shipped at a temperature of 5 C to the Caine Veterinary Teaching Center (CVTC), University of Idaho, Caldwell, Idaho, USA.

Laboratory analysis

After arriving at the CVTC, the swabs were removed from the transport media and inoculated onto nonselective Columbia blood agar (Becton-Dickinson and Company, Sparks, Maryland, USA) and onto selective Columbia blood agar containing antibiotics (Becton-Dickinson) that inhibit growth of non-Pasteurellaceae (Jaworski et al., 1993). Plates were incubated at 37 C in 5–10% carbon dioxide atmosphere and inspected after 24 and 48 hr of incubation. Based on morphology, suspect colonies were chosen for further identification. There was no limitation on the selection and retention of types and number of colonies per plate. Bacterial isolates identified as *P. trehalosi* and *M. haemolytica* were evaluated for the presence of hemolysis on blood agar and were classified using previously described biogrouping (Bisgaard and Mutters, 1986; Bisgaard et al., 1986) and biovariant systems (Jaworski et al., 1998).

Data analysis

Because of the limited number of sampled animals and the large number of possible biovariant categories, the power of the study to detect differences among the three study populations was low. To improve the power of the statistical analysis, all the biovariant categories for isolates were collapsed to biogroup categories. Because multiple isolates with identical biovariants were potentially obtained for each animal sample and to minimize problems of nonindependence within a biogroup category, only a single biovariant per animal was allowed to populate a unique biogroup category. However, if multiple biovariants within a biogroup had different hemolysis phenotypes, a biovariant representing each hemolysis pattern was retained for the analysis.

For each bacterial species, isolate biogroups were cross-classified against each population of sampled sheep and the distributions tested for homogeneity using Fisher's exact test for $i \times j$ tables (Agresti, 2002). Biogroup prevalence for each bacterial species-biogroups, stratified by sheep population, was calculated.

TABLE 2. Number and type of bacteria isolated from oropharyngeal swabs taken from SNBS, WMBS, and DS in a study of three populations in California (2001–06).[a]

Bacteria	SNBS	WMBS	DS
Mannheimia haemolytica	35	18	49
Pasteurella trehalosi	81	27	12
Other bacteria	5	ND	6
No bacteria detected	6	0	0
Totals	129	45	67

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep; ND = not detected.

Using DS as the comparison, prevalence odds ratios (pOR) and 95% exact confidence limits for SNBS and WMBS by biogroups were computed. For *M. haemolytica*, a biogroup that was common (and similar in prevalence) for all three populations was chosen as the reference. Fisher's exact test was used to test for differences in the *P. trehalosi* biogroup distribution between wild and domestic sheep and in the hemolytic activity of the isolates. Finally, odds ratios were calculated to further evaluate differences among study populations. *P*-values$<$0.05 were considered statistically significant.

RESULTS

The focus of this study was on the occurrence and distribution of *P. trehalosi* and *M. haemolytica* in three unique populations of domestic (DS) and bighorn sheep (SNBS and WMBS). Animals were captured and sampled using a different sampling strategy for the three populations. Between 2001 and 2006, 78 SNBS were sampled; between 2004 and 2005, 22 WMBS were sampled, and in 2006, 30 DS were sampled. Although animals could have been sampled more than once during the study period with multiple identical isolates with the same biovariant recovered from the same animal, only a single sample was chosen when an animal had been captured twice, and a single isolate was selected when multiple isolates with identical hemolysis were recovered from the same animal and represented the same biovariant. Among the sampled animals, *M. haemolytica* represented 27% (35 of 129) of the isolates from SNBS, 40% (18 of 45) from WMBS, and 73% (49 of 67) from DS. *Pasteurella trehalosi* represented 63% (81 of 129) of the isolates from SNBS, 60% (27 of 45) from WMBS, and 18% (12 of 67) from DS (Table 2).

Mannheimia haemolytica: *Mannheimia haemolytica* was isolated from animals in all three sampled populations during all the sampling periods. A total of 102 isolates were recovered, which represented 43 biovariants and 11 biogroups (Tables 3 and 4). *Mannheimia haemolytica*, recovered from DS, showed the greatest diversity, with 25 biovariants and 10 biogroups. In contrast, 14 biovariants in five biogroups and 10 biovariants in six biogroups were recovered from SNBS and WMBS, respectively. For all subsequent analyses, only biogroup affiliation was considered.

The *M. haemolytica* biogroups were differentially distributed among the three sampled populations ($P<0.001$), with each sheep population having a distinct array of biogroups. Statistically significant differences were detected between WMBS and DS, between SNBS and DS, and between WMBS and SNBS ($P<0.001$). This is explained by three biogroups (biogroups 5, 9, and 11) unique only to DS and one biogroup (biogroup 2) unique to SNBS. Despite these overall differences, the distribution of the shared biogroups was more similar between WMBS and DS than between SNBS and DS, as demonstrated by the calculated pORs (Table 5). Of the seven biogroups that were shared

TABLE 3. Biovariants classification of *Mannheimia haemolytica* isolated from oropharyngeal swabs obtained from SNBS, WMBS, and DS.[a]

M. haemolytica biovariants[b]	SNBS	WMBS	DS
1	.[c]	6	3
1^{α}	.	.	1
1^{B}	.	.	2
$1^{\alpha E}$	.	.	1
2	1	.	.
3	7	1	2
3^{α}	8	1	.
$3^{\alpha B}$	1	.	.
$3^{\alpha CD}$	1	.	.
3^{B}	3	.	.
$3^{\beta CB}$	1	.	.
3^{D}	1	.	.
5	.	.	4
5^{B}	.	.	3
5^{D}	.	.	1
7	1	.	.
7^{B}	.	.	1
7^{BX}	.	.	1
8	.	1	2
9^{α}	.	.	1
10	.	3	2
10^{α}	3	2	.
$10^{\alpha B}$	2	.	.
$10^{\alpha C}$	2	.	.
11	.	.	10
11^{X}	.	.	1
16	.	.	1
16^{B}	.	.	2
$16^{\alpha BE}$	.	1	.
$16^{\alpha E}$	.	.	2
U^{α}	.	.	3
$U^{\alpha\beta}$	.	1	.
$U^{\alpha B}$	.	.	1
$U^{\alpha C}$	.	.	1
$U^{\alpha\beta B}$	.	.	1
$U^{\alpha BX}$	.	1	.
$U^{\alpha\beta BX}$	.	1	.
$U^{\alpha\beta X}$	.	.	1
$U^{\alpha R}$	.	.	1
U^{β}	1	.	.
$U^{\beta B}$	3	.	1
Totals	35	18	49

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

[b] Biogroup numbers without superscript letters indicate isolates belong to biogroups previously described (Bisgaard et al., 1986). Superscript capital and greek letters indicate biovariant kinds previously described (Jaworski et al., 1998).

[c] · = Not detected.

among at least two of the study populations, three were not observed in SNBS, two were strongly dominated by SNBS ($pOR_{biogroup3}$=21.0, 95% confidence interval [CI]=2.52–242.68; $pOR_{biogroup10}$= 7.0, 95% CI=0.73–90.81), one was observed only once (biogroup 7), and one (biogroup U) was common in all three populations. In contrast, all but one of the shared biogroups (biogroup 7) was observed in WMBS, and the pORs (with the exception of biogroup 10) were unremarkable. The power for these comparisons is poor (reflected in the large confidence intervals) because only 18 isolates were collected from the WMBS population. There were no differences in the prevalence of *M. haemolytica* hemolysis among the three populations (P=1.00).

Pasteurella trehalosi: Similar to *M. haemolytica*, *P. trehalosi* was isolated from animals in all three sampled populations at all the time periods (Table 2). A total of 120 isolates were recovered and represented 12 biovariants and 2 biogroups. Eight, five, and six biovariants were recovered from SNBS, WMBS, and DS, respectively. In addition, although both biogroups were recovered in SNBS and WMBS, only one biogroup was detected in DS (Tables 6 and 7). *Pasteurella trehalosi* biogroups were differentially distributed among the three sampled populations (P<0.001). Although there were differences in biogroup distribution among WMBS and both DS and SNBS (P=0.013), there was no difference in the distribution of isolates by biogroups between SNBS and DS (P=1.00). The differences detected between WMBS and the other two populations were probably a consequence of biogroup 4 being present in the highest proportion in the WMBS. *Pasteurella trehalosi* hemolysis was different when comparing isolates from the three sheep populations (P<0.001). No hemolytic isolates were recovered from SNBS. The prevalence of hemolytic isolates from WMBS was intermediate between SNBS

TABLE 4. Biogroup classification and corresponding proportions and hemolysis pattern of *Mannheimia haemolytica* isolated from oropharyngeal swabs obtained from SNBS, WMBS, and DS.[a,b]

	SNBS			WMBS			DS			
Biogroups	B+	B−	Subtotals	B+	B−	Subtotals	B+	B−	Subtotals	Totals
1	.[c]	.	.	6	.	6 (0.33)	6	.	6 (0.13)	12
2	1	.	1 (0.03)	.	.	.	.	.	.	1
3	19	2	21 (0.62)	2	.	2 (0.11)	2	.	2 (0.04)	25
5	.	.	.	.	.	.	8	.	8 (0.18)	8
7	1	.	1 (0.03)	.	.	.	1	1	2 (0.04)	3
8	.	.	.	1	.	1 (0.05)	.	2	2 (0.04)	3
9	.	.	.	.	.	.	1	.	1 (0.02)	1
10	6	1	7 (0.21)	5	.	5 (0.28)	2	.	2 (0.04)	14
11	.	.	.	.	.	.	10	.	10 (0.22)	10
16	.	.	.	1	.	1 (0.05)	1	3	4 (0.09)	5
U	.	4	4 (0.12)	.	3	3 (0.17)	5	3	8 (0.18)	15
Totals	27	7	34	15	3	18	36	9	45	97

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

[b] B+ = presence of hemolysis; B− = absence of hemolysis.

[c] · = Not detected.

and DS, with the isolates recovered from WMBS less likely to be hemolytic compared with isolates from DS (pOR=0.11, 95% CI=0.01–0.64).

DISCUSSION

The objective of this study was to further evaluate the relationship of pneumonia-related mortality in bighorn sheep and the bacteria (specifically, *P. trehalosi* and *M. haemolytica*) endemic to domestic sheep. Specifically, this field-based study evaluated the hypothesis that a bighorn sheep population with a recent history of pneumonia (WMBS) would have a similar distribution of oropharyngeal-derived *P. trehalosi* and *M. haemolytica* to those recovered from domestic sheep (DS). This was contrasted to the bacterial species recovered from a population of bighorn sheep (SNBS) without a recent history of pneumonia. The data from this study came from healthy animals that were captured, restrained, and subsequently, sampled to isolate bacteria from the oropharynx. Although some of the findings from the bacterial characterizations were consistent with the study's hypothesis, most of the data do not support the study hypothesis.

TABLE 5. Prevalence odds ratio and 95% confidence intervals for the presence of *Mannheimia haemolytica* biogroups SNBS and WMBS compared with DS.[a]

	SNBS		WMBS	
Biogroup	pOR	95% CI	pOR	95% CI
1	.[b]	.	2.67	0.35–22.77
3	21	2.52–242.68	2.67	0.13–50.85
7	1	0.01–25.35	.	
8	.		1.33	0.02–35.44
10	7	0.73–90.81	6.67	0.57–96.61
16	.		0.67	0.01–12.39
U	Reference	.	Reference	.

[a] pOR = Prevalence odds ratio; 95% CI = 95% confidence intervals; SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

[b] · = Not detected.

TABLE 6. Biovariant classification of *Pasteurella trehalosi* isolated from oropharyngeal swabs obtained from SNBS, WMBS, and DS.[a]

P. trehalosi biovariants[b]	SNBS	WMBS	DS
2	66	15	7
2^{B}	1	.[c]	1
2^{C}	2	1	1
2^{CE}	.	.	1
2^{D}	2	.	.
2^{E}	2	.	1
2^{ES}	1	.	.
2^{GS}	.	.	1
2^{S}	6	1	.
4	1	.	.
4^{C}	.	1	.
4^{CDS}	.	9	.
Totals	81	27	12

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

[b] Biogroup numbers without superscript letters indicate isolates belong to biogroups previously described (Bisgaard et al., 1986).

[c] · = Not detected.

Based on the relative proportion of *P. trehalosi* and *M. haemolytica* recovered from the three sheep populations, there was more similarity between the two bighorn sheep populations than with the DS population. *Pasteurella trehalosi* represented a small proportion of the isolates in DS, whereas they represented an important portion of the oropharyngeal bacterial population in both SNBS and WMBS. This trend has been observed in other studies, which also note that *P. trehalosi* is more commonly found in bighorn sheep populations and rarely found in DS populations (Ward et al., 1990; Jaworski et al., 1998).

For *M. haemolytica*, the data suggest that the biogroups observed in the SNBS population were different from those isolated from the DS population, whereas, the in-common biogroups recovered from the WMBS appeared more similar to those from the DS population. Field observations show a positive correlation between pneumonia-associated mortality in bighorn sheep and proximity to domestic sheep grazing on nearby pastures (Martin et al., 1996). This is also observed in experimental studies, where bighorn sheep are more susceptible to Pasteurellaceae strains carried by healthy domestic sheep and can develop pneumonia once infected (Onderka et al., 1988). However, the confidence intervals of the pOR that were calculated in our study were very wide (particularly for the WMBS estimates) because of the small sample size and the low power of the statistical test. The consequent type II error was high, making support of the theory that biogroup distribution would be more similar between WMBS and DS compared with SNBS and DS less certain. Consequently, the ecologic hypothesis will need to be evaluated using a larger sample within the populations and with expansion of the study to other populations. It should be noted that biogroup 1, which has been associated with disease in domestic ruminants (Jaworski et al., 1998), was not isolated from SNBS but was isolated from

TABLE 7. Biogroups classification and corresponding proportions and hemolysis pattern of *Pasteurella trehalosi* isolated from oropharyngeal swabs obtained from SNBS, WMBS, and DS.[a]

	SNBS			WMBS			DS			
Biogroups	B+	B−	Subtotals	B+	B−	Subtotals	B+	B−	Subtotals	Totals
2	.[b]	68	68 (0.98)	4	11	15 (0.60)	9	3	12 (1)	95
4	.	1	1 (0.01)	2	8	10 (0.40)	.	.	.	11
Totals	.	69	69	6	19	25	9	3	12	106

[a] SNBS = Sierra Nevada bighorn sheep; WMBS = White Mountains bighorn sheep; DS = domestic sheep.

[b] · = Not detected.

both WMBS and DS. Moreover, all of the biogroup 1 isolates were hemolytic.

We had hypothesized that β-hemolysis might be an indication of virulence because it is often associated with the presence of the LKT structural gene, *lktA* (Fisher et al., 1999). Leukotoxin is considered an important element in the etiology of bacterial pneumonia in domestic ruminants and bighorns. However, the approximate equal prevalence of β-hemolytic *Mannheimia* isolates in the three populations indicates that, at least in the present study, β-hemolytic *Mannheimia* is clearly not a sufficient cause in the disease process at an ecologic level.

There was lower biogroup variability in *P. trehalosi* compared with *M. haemolytica*, with only two biogroups detected in the complete isolate set. Biogroup 2 was recovered from all three sheep populations, with biogroup 4 uniquely recovered from WMBS. The distribution of biogroup 4 isolates to a single sheep population is consistent with the previous literature reporting biogroup 2 as predominant in domestic and bighorn sheep samples compared with biogroup 4 (Jaworski et al., 1998). The specificity of biogroup 4 to this population of WMBS is consistent with a recent article that reported that *P. trehalosi* tends to be more host specific than *M. haemolytica* and that strains of *P. trehalosi* can be regionally limited (Kelley et al., 2007).

The prevalence of hemolysis in *P. trehalosi* was different among the three populations. No hemolytic *P. trehalosi* were recovered from SNBS. In contrast, a high proportion of the *Pasteurella* isolates from DS were hemolytic. The prevalence of hemolysis in *P. trehalosi* recovered from WMBS was intermediate between SNBS and DS. These data are consistent with the previous literature showing that it is common to isolate nonhemolytic *P. trehalosi* from healthy bighorn sheep (Onderka et al., 1988; Wild and Miller, 1991; Snipes et al., 1992).

Debate continues about the role of the bacteria characterized in our study in the occurrence of pneumonia in the bighorn sheep population. The presence of *Pasteurella* and *Mannheimia* isolates in our sampled healthy sheep, including the SNBS population, which had not recently experienced pneumonia-related mortality, supports the concept that these bacteria may be opportunistic bacteria and that other etiologic agents are the primary cause of pneumonia in the WMBS (Spraker et al., 1984). Moreover, the literature reports that pasteurellosis-associated bighorn sheep die-offs occur that are not related to the presence of domestic sheep (Ward et al., 1997) and are likely caused from environmental stress, facilitating the commensal host-specific *Pasteurella* and *Mannheimia* to invade the lower respiratory tract, resulting in pneumonia (Spraker et al., 1984). In domestic ruminants, pneumonic pasteurellosis is also considered an opportunistic infection rather than a primary disease (Whiteley et al., 1992).

Pulmonary clearance mechanisms may be impaired by any of several different causes, but primary infectious agents, such as respiratory viruses and, possibly, *Mycoplasma ovipneumoniae*, are frequently incriminated (Brodgen et al., 1998). Wild sheep may also harbor these pathogenic agents, as has been demonstrated with *M. ovipneumoniae* (Black et al., 1988; Besser, 2007; Besser et al., 2008).

There is ample evidence in the literature that bighorn sheep populations have diminished following the arrival of domestic sheep in pastures associated with their range. This appears also to be related with pneumonia-associated mortality that has been associated with *Pasteurella* and *Mannheimia* infections as primary pathogens, which are hypothesized to originate from domestic sheep. The data from our ecologic study did not strongly support that hypothesis.

Our study was focused on sampling healthy animals from bighorn sheep populations with different histories of pneumonia-related mortality. Although we observed that biotypes of *M. haemolytica*

recovered from SNBS (with no recent history of pneumonia-associated mortality) were dissimilar from those recovered from DS, the evidence that the *M. haemolytica* biogroups from WMBS (a population with recent history of pneumonia-associated mortality) were similar to DS biogroups was weak. The evidence from the *P. trehalosi* isolates and prevalence of hemolysis suggest little difference among the three sheep populations.

Our study did not evaluate the isolates from cases of respiratory disease in WMBS, and we assume that the Pasteurellaceae identified are either commensal or transient pharyngeal organisms in bighorn and domestic sheep. Moreover, it is not clear the significance of β-hemolysis as an indicator of virulence in field isolates. The possibility that the bacteria we studied are mainly secondary pathogens suggests that studies should be designed to more closely evaluate biologic and environmental factors that coincide to cause pneumonia in bighorn sheep. One approach would be to evaluate other bacterial or viral species using culture-independent methods that have become more prevalent.

ACKNOWLEDGMENTS

We acknowledge T. Stephenson and V. Bleich and the biologists of the California Department of Fish and Game Sierra Nevada Bighorn Sheep Program for providing the opportunity for field sample collection and for overall support for this project.

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Received for publication 13 July 2008.