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SUSCEPTIBILITY OF DALL SHEEP (*OVIS DALLI DALLI*) TO PNEUMONIA CAUSED BY *PASTEURELLA HAEMOLYTICA*

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ABSTRACT: We evaluated susceptibility of Dall sheep (*Ovis dalli dalli*) to bacterial pneumonia induced by two strains of *Pasteurella haemolytica* of domestic sheep origin by evaluating the sensitivity of blood neutrophils of eight Dall sheep to lysis by cytotoxins of *P. haemolytica*, and by intratracheal inoculation of three Dall sheep, two bighorn sheep (*Ovis canadensis*), and two domestic sheep with 3.7×10^6 or 2.5×10^7 colony forming units of *P. haemolytica*. Neutrophils from the Dall sheep were more sensitive to lysis by cytotoxins from supernatants of a *P. haemolytica*, biotype A, serotype 2 (A2), of domestic sheep origin, than were neutrophils from six bighorn sheep. This cytotoxic bacterium was the same isolate that was used for intratracheal inoculation of two Dall sheep and two domestic sheep. Inoculation of this cytotoxic *P. haemolytica* A2 resulted in fatal fibrinopurulent pleuropneumonia in the first Dall sheep within 24 hr of inoculation, and pneumonic lesions in the second Dall sheep before it was euthanized 52 hr after inoculation. This strain of *P. haemolytica* A2 did not cause respiratory disease when inoculated into two domestic sheep. A noncytotoxic strain of *P. haemolytica*, biotype T, serotype 3,4,10 of domestic sheep origin did not result in pneumonia in the third Dall sheep or two bighorn sheep. Prior to inoculation, *P. haemolytica*, biotype T isolates were obtained from all three Dall sheep, but none of these isolates was cytotoxic. At necropsy, cytotoxic *P. haemolytica* A2 was isolated from lungs and other tissues of the two pneumonic Dall sheep. Based on these results, we conclude that Dall sheep appear to be at least as sensitive as bighorn sheep to pneumonia caused by *P. haemolytica* A2 of domestic sheep origin. Because in vitro and in vivo results appear closely correlated in this and other studies, we believe with additional evaluation and standardization, neutrophil cytotoxicity tests may serve as a substitute for live animal challenges in future studies of pathogenic *P. haemolytica* in wild sheep.

Key words: Dall sheep, *Ovis dalli dalli*, bighorn sheep, *Ovis canadensis*, *Pasteurella haemolytica*, pneumonia, cytotoxicity, experimental study.

INTRODUCTION

A variety of mortality factors affecting Dall sheep (*Ovis dalli dalli*) populations have been documented (Bowyer and Leslie, 1992). Pneumonia, caused primarily by *Pasteurella haemolytica*, is the major mortality factor in free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in North America (Foreyt et al., 1994), but to our knowledge has not been documented in free-ranging Dall sheep. Under controlled experimental conditions, close contact between healthy bighorn sheep and domestic sheep resulted in fatal pneumonia caused by *P. haemolytica* in the bighorn sheep (Foreyt, 1989), and intratracheal inoculation of some strains of *P. haemolytica* from domestic sheep was lethal in bighorn sheep (Foreyt et al., 1994). Dall sheep are indigenous to Alaska (USA) and Canada in areas where exposure to domestic sheep is unlikely. However, the presence of domestic sheep in some of these areas could result in direct contact and transfer of pathogens (Heimer et al., 1992). Pneumonia caused by *Mycoplasma ovipneumoniae* has occurred in captive Dall sheep in a zoo in Canada (Black et al., 1988), but susceptibility of free-ranging Dall sheep to pneumonia caused by *P. haemolytica* has not been investigated. Our purpose was to evaluate susceptibility of blood neutrophils from Dall sheep to lysis when exposed to cytotoxins from previously identified cytotoxic and noncytotoxic strains of *P. haemolytica*, and compare the degree of lysis with bighorn sheep neutrophils. A second objective was to evaluate the susceptibility of Dall sheep to a strain of *P. haemolytica* from domestic sheep that previously resulted in fatal acute pneumonia in exper-

imentally inoculated bighorn sheep (Foreyt et al., 1994).

MATERIALS AND METHODS

The in vitro neutrophil cytotoxicity experiments were done between July 1993 and May 1995. The in vivo inoculation experiment was done in July 1993, and involved three Dall sheep, two Rocky Mountain bighorn sheep, and two domestic sheep. All animals used in all experiments were clinically healthy. Blood from Dall sheep for the neutrophil sensitivity experiments was obtained from eight Dall sheep from three locations. Three Dall sheep were from the Metropolitan Toronto Zoo, West Hill, Ontario, Canada, and consisted of three yearling males with unrelated mothers and an unknown paternal history. They were transported to Washington State University (WSU), Pullman, Washington (USA), 7 days before the blood samples were collected. Four unrelated Dall sheep, consisting of a 10-yr-old female and three 9-mo-old male lambs, were from the Calgary Zoo, Calgary, Alberta, Canada; an additional yearling male Dall sheep from the Alaska Zoo, Anchorage, Alaska, was born in captivity from a mother that was originally captured in the wild. Blood samples also were obtained from six bighorn sheep, including three 6-mo-old bighorn sheep male lambs, two adult males, and one adult female. All bighorn sheep were born in captivity at WSU. Their mothers were all different and had been captured in the wild several years before, and their father also had been wild captured from the same herd as the mothers.

For the inoculation experiment, three Dall sheep yearling rams were obtained from the Metropolitan Toronto Zoo on 7 July 1993, transported to WSU and placed in a 4 × 7 m isolation facility with concrete floors. These Dall sheep were three of the eight Dall sheep used in the neutrophil cytotoxicity experiment. Dall sheep were fed alfalfa hay ad libitum, and water and mineralized salt were available at all times. The two bighorn sheep were yearling rams that had been captured as free-ranging lambs on Hall Mountain in northeastern Washington (48°50′N 117°15′W) and maintained in a 1-ha outdoor enclosure for 7 mo before use in this experiment. Feed consisted of pasture grasses, supplemented with alfalfa hay. Water and mineralized salt were available at all times. The two domestic sheep were 1-yr-old castrated males and were maintained in a separate 1 ha-pasture. Feed consisted of pasture grasses, supplemented with alfalfa hay. Water and mineralized salt were available at all times.

Peripheral blood samples from eight Dall sheep, and six bighorn sheep were collected by jugular venipuncture into citrate phosphate dextrose solution (Sigma Chemical Company, St. Louis, Missouri, USA). Blood was maintained at 4 C until used in the cytotoxicity assay within 24 hr of collection (Silflow et al., 1994). Following centrifugation at 850 × *G* for 15 min, the plasma and buffy coats were discarded. Hypotonic lysis of red cells was accomplished by the addition of 45 ml distilled water for 50 sec followed by the addition of 5 ml of 10× phosphate buffered saline. Following centrifugation at 600 × *G* for 10 min, the lysis and centrifugation steps were repeated, and the final cell pellets were resuspended in Hanks balanced salt solution (HBSS) (Gibco Laboratories, Grand Island, New York, USA) containing 1% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, Utah, USA). Cells were quantitated using a hemocytometer (American Optical Corporation, Buffalo, New York), and cell viability was determined by trypan blue exclusion (Boyse et al., 1964). Typical yields were >90% neutrophils, and these cells had >90% viability. For each experiment, cells were adjusted to a concentration of 5×10^6 cells/ml in HBSS and 1% FBS.

Cytotoxins were isolated from *P. haemolytica* culture supernatants using the method of Silflow et al. (1993). Individual *P. haemolytica* isolates obtained from all species used in the experiments were streaked onto 5% blood agar plates (Beckton Dickinson Microbiological Systems, Cockeysville, Maryland, USA) and incubated for 18 hr at 37 C. A negative control bacterium, *Enterobacter cloacae* (American Type Culture Collection No. 35030, Rockville, Maryland), was handled identically. Several morphologically similar colonies were used to inoculate 100 ml of brain-heart infusion broth (Difco Laboratories, Detroit, Michigan, USA) which was incubated at 37 C until cultures reached early logarithmic growth. To quantitate the number of bacteria, the optical densities (OD) of the cultures were measured at a wavelength of 600 nm (OD_{600}) until 1 OD_{600} (8×10^8 bacteria/ml) was reached (Maniatis et al., 1982). Bacteria were centrifuged for 10 min at 6,000 × *G* to a pellet, and resuspended in 30 ml of RPMI-1640 media (Gibco Laboratories) containing 7% FBS. Following incubation for 1 hr at 37 C, the bacteria again were centrifuged at 6,000 × *G* for 10 min, and the culture supernatants were removed and filter sterilized in a 0.45 μm filter (Sigma Chemical Company). Culture supernatants were dialyzed to exhaustion against distilled water and lyophilized.

We characterized the relative potency of toxins produced by various *P. haemolytica* isolates by adding bacterial culture supernatants to

neutrophils in vitro. Neutrophils from every animal were tested with every supernatant. Cytotoxicity was quantitated by assessing the release of lactate dehydrogenase (LDH) from neutrophils into the culture medium (Korzeniewski and Callewaert, 1983). Cytotoxicity was determined at final concentrations of supernatant of 150, 100, 50, 5, and 0.5 μg of crude lyophilized toxin/50 μl HBSS supernatant which contained 1% FBS (Shewen and Wilkie, 1982). All of the samples were resuspended in HBSS containing 1% FBS prior to the assay. Fifty μl of each supernatant preparation containing cytotoxin was added to the wells of 96-well plates, followed by the addition of 2.5 $\times$ 10^5 neutrophils in 50 μl of HBSS containing 1% FBS to each well. Following 1 hr incubation at 37 C, 100 μl of LDH substrate was added. Quantitation of the reduced LDH substrate was based on a Titertek 96-well plate reader (Flow Laboratories, McLean, Virginia, USA) coupled to an on-line IBM-XT computer (International Business Machines, Boca Raton, Florida, USA). All samples were compared to neutrophils treated with a 0.5% solution of the detergent Triton-X (Sigma Chemical Company) (maximal release) and untreated cells (background release) and the results recorded as a percentage of LDH released from detergent-treated cells. The potency of the various cytotoxins was determined from the 50% lethal concentration (LC_{50}), represented by the graphic intersection of cytotoxin concentration and 50% neutrophil death (Silflow et al., 1993) with a curve fitted to the graph (Fig. 1).

In the neutrophil cytotoxicity experiment, neutrophils from eight Dall sheep were compared with neutrophils from six bighorn sheep for relative sensitivity to supernatants of a known cytotoxic isolate of *P. haemolytica*, biotype A, serotype 2 (hereafter referred to as *P. haemolytica* A2), and a known noncytotoxic isolate of *P. haemolytica* biotype T, serotype 4, obtained from a healthy bighorn sheep (Tables 1 and 2). The cytotoxic *P. haemolytica* A2 was obtained from a healthy domestic sheep and was the same isolate used in the animal inoculation studies (Table 1).

In the inoculation experiment, supernatants from 26 isolates of *P. haemolytica* from Dall sheep (n = 14), bighorn sheep (n = 4), and domestic sheep (n = 8) were tested with neutrophils from three bighorn sheep for sensitivity to lysis (cytotoxicity). Cytotoxicity was defined as causing >50% neutrophil death as measured by release of lactate dehydrogenase into culture supernatant. The isolates tested were collected from each of the species before and after experimental inoculation (Table 1). Results were tested for statistical significance

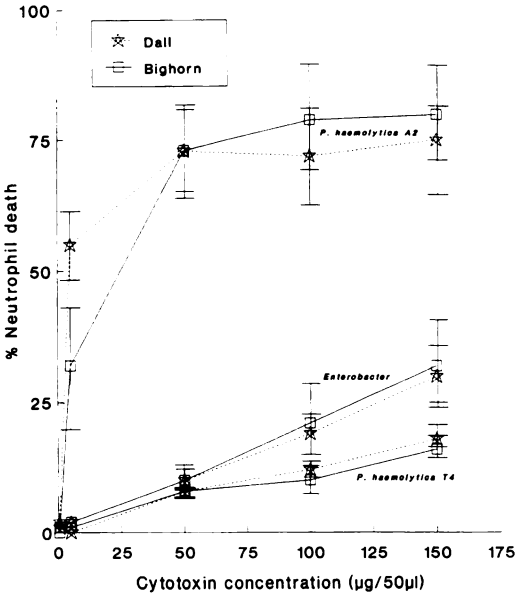

FIGURE 1. Dose response curves of supernatants from bacterial isolates tested for cytotoxicity with neutrophils from either Dall sheep (Dall) (n = 8) or bighorn sheep (Bighorn) (n = 6); A2 = cytotoxic *P. haemolytica* A2 from a healthy domestic sheep; T4 = noncytotoxic *P. haemolytica* T4 from a healthy bighorn sheep, and *Enterobacter* = a noncytotoxic control bacterium (*Enterobacter cloacae*). Data points are mean ± SE.

using the statistical software package Statistix version 3.5 (Analytical Software, St. Paul, Minnesota, USA) by first performing analysis of variance (ANOVA), then testing for differences between means using least squared differences (LSD) ($P < 0.05$).

Two isolates of *P. haemolytica* from healthy domestic sheep were used as separate experimental inocula. One cytotoxic *P. haemolytica* A2 isolate, previously characterized by ribotyping, was lethal in experimentally infected bighorn sheep (Foreyt et al., 1994). The second isolate was *P. haemolytica* biotype T, which agglutinated in antisera to serotypes 3,4, and 10, and was noncytotoxic (nonpathogenic) (Sweeney et al., 1994). The *P. haemolytica* isolates were preserved in phosphate buffered glycerol at −70 C. They were grown in brain heart infusion broth and numbers of colony forming units (CFU) for inoculation were estimated by methods described by Foreyt et al. (1994). While animals were restrained physically, the bacterial suspensions were injected intratracheally with a 3 cc syringe and 20 gauge needle. All animals were inoculated on one day (day 0) with 3.7 $\times$ 10^6 or 2.5 $\times$ 10^7 CFU of *P.*

TABLE 1. Cytotoxicity, expressed as percent neutrophil death, of bacterial isolates to neutrophils of eight Dall sheep (*Ovis dalli dalli*).

	Enterobacter cloacae Cytotoxin concentration (μg/50 μl)					*Pasteurella haemolytica* A2[a] Cytotoxin concentration (μg/50 μl)					*Pasteurella haemolytica* T4[b] Cytotoxin concentration (μg/50 μl)				
Animal	150	100	50	5	0.5	150	100	50	5	0.5	150	100	50	5	0.5
1	18[c]	14	4	2	3	100	100	100	73	4	16	8	10	0	2
2	12	11	6	0	1	100	100	100	60	3	12	11	7	0	4
3	26	15	8	5	0	100	100	100	73	5	20	12	10	0	0
4	32	22	11	3	0	66	61	65	46	0	16	14	7	1	0
5	31	20	9	0	0	46	42	44	35	0	15	11	6	0	0
6	50	28	14	3	0	64	57	61	58	0	22	14	10	1	0
7	44	30	17	3	0	59	56	57	58	0	20	16	7	1	0
8	24	10	14	0	0	64	58	56	36	0	23	6	9	0	0
Mean	30	19	10	2	1	75	72	73	55	2	18	12	8	0	1
SE[d]	4.5	2.7	1.6	0.7	0.4	7.7	8.5	8.2	5.2	0.8	1.4	1.2	0.6	0	0.5

[a] Domestic sheep origin.
[b] Bighorn sheep origin.
[c] Mean percentage of neutrophil death of three replicates at specified cytotoxin concentration.
[d] Standard error of the mean.

haemolytica (Table 1). Before inoculation, pharyngeal swabs were collected from all animals and transported to the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington, for bacterial and viral isolations by methods described by Foreyt et al. (1994). Swabs for bacterial isolations were streaked onto 5% sheep blood agar plates (Becton Dickinson Microbiological Systems) within 2 hr of collection. Subsequent pharyngeal swab samples from surviving animals were collected 22 days after inoculation. Methods for isolation and identification of viruses (Foreyt et al., 1994) and biotypes and serotypes of *P. haemolytica* isolates (Foreyt and Lagerquist, 1994) have been described previously. Fecal samples from the Dall sheep and bighorn sheep were evaluated for lungworm larvae by a modified Baermann technique (Beane and Hobbs, 1983). After inoculation, animals were observed three times daily for signs of disease, and dead animals were submitted to WADDL for complete necropsy evaluation. Two Dall sheep were euthanized with an intravenous injection of 30 g of sodium pentabarbital (Anthony Products Company, Arcadia, California, USA) 52 hr after inoculation. At necropsy, bacterial and viral isolations were attempted from tissues including tonsil, bronchial lymph nodes, spleen, and lungs. Representative tissues including lung, liver, bronchial lymph nodes, adrenal gland, cerebrum, brainstem, cerebellum,

TABLE 2. Cytotoxicity, expressed as percent neutrophil death, of bacterial isolates to neutrophils of six bighorn sheep (*Ovis canadensis canadensis*).

	Enterobacter cloacae Cytotoxin concentration (μg/50 μl)					*Pasteurella haemolytica* A2[a] Cytotoxin concentration (μg/50 μl)					*Pasteurella haemolytica* T4[b] Cytotoxin concentration (μg/50 μl)				
Animal	150	100	50	5	0.5	150	100	50	5	0.5	150	100	50	5	0.5
1	15[c]	10	10	2	1	100	100	78	4	1	12	10	6	1	0
2	17	9	7	0	0	100	100	100	14	0	11	4	8	0	1
3	15	8	5	3	3	100	100	85	7	1	16	6	5	0	2
4	65	42	14	3	0	60	55	61	60	0	18	14	9	2	0
5	42	30	12	5	0	63	63	62	58	0	20	15	10	2	0
6	39	24	9	1	0	54	55	54	49	0	17	13	7	1	0
Mean	32	21	10	2	1	80	79	73	32	0	16	10	8	1	1
SE[d]	8.2	5.7	1.3	0.7	0.5	9.2	9.5	7.1	10.8	0.2	1.4	1.8	0.8	0.4	0.3

[a] Domestic sheep origin.
[b] Bighorn sheep origin.
[c] Mean percentage of neutrophil death of three replicates at specified cytotoxin concentration.
[d] Standard error of the mean.

TABLE 3. Summary of *Pasteurella haemolytica* isolated from animals in the inoculation experiment.

Animal number	Inoculum			Preinoculation isolates[a]		Postinoculation isolates[a]		
	Cfu[b]	Biotype/ serotype	Cyto- toxic	Biotype/ serotype	Cyto- toxic	Biotype/ serotype	Cyto- toxic	Pneu- monia
Dall sheep 1	2.5×10^7	A/2	+	T/3, 4, 10	−	A/2[c]	+	+
				A/Untypeable	−	T/3, 4, 10[d]	−	
Dall sheep 2	3.7×10^6	A/2	+	T/3, 4, 10	−	A/2[c]	+	+
				T/3, 4, 10	−	T3, 4, 10[e]	−	
Dall sheep 3	2.2×10^7	T/3, 4, 10	−	T/Untypeable	−	Negative	NA[f]	−
				T/3, 4, 10	−			
Bighorn sheep 1	2.2×10^7	T/3, 4, 10	−	T/3, 4	−	ND[g]	NA	−
				A/Untypeable	+			
Bighorn sheep 2	2.2×10^7	T/3, 4, 10	−	T/Untypeable	−	ND	NA	−
				T/3, 4, 10	−			
Domestic sheep 1	3.7×10^6	A/2	+	T/Untypeable	+	T/3, 4	+	−
				A/Untypeable	+	T/Untypeable	−	
						A/11	+	
Domestic sheep 2	2.5×10^7	A/2	+	T/3, 4, 10	+	T/3, 4	−	−
						T/Untypeable	−	

[a] From pharyngeal swabs unless noted.
[b] Colony forming units.
[c] Isolated from bronchial lymph node and lung.
[d] Isolated from bronchial lymph node, lung and tonsil.
[e] Isolated from tonsil.
[f] Not applicable.
[g] Not done.

thyroid gland, pituitary gland, tongue, spleen, kidney, testicle, epididymis, and abomasum were fixed in 10% buffered formalin, sectioned at 5 μm, and stained in hematoxylin and eosin for microscopic evaluation.

RESULTS

The neutrophils from Dall sheep were more sensitive to lysis at the 50% lethal concentration by cytotoxic *P. haemolytica* A2 than were neutrophils from bighorn sheep (4 μg/50ml vs. 24 μg/50ml; Tables 1 and 2; Fig. 1), but the difference was not statistically significant ($P > 0.05$). *Pasteurella haemolytica* T4 was not cytotoxic at any cytotoxin concentration (Fig. 1). Mean percent neutrophil death of Dall sheep and bighorn sheep neutrophils was significantly greater ($P < 0.01$) when exposed to cytotoxins from *P. haemolytica* A2 than *E. cloacae* or *P. haemolytica* T4 at the 5, 50, 100 and 150 μg/50 μl concentrations (Fig. 1). *Enterobacter cloacae* cytotoxins also resulted in significantly greater ($P < 0.01$) neutrophil death when compared to *P. haemolytica* T4 at the 5, 50, 100, and 150 μg/μl concentrations (Fig. 1).

None of the six *P. haemolytica* isolates obtained from preinoculation pharyngeal swabs of three Dall sheep were cytotoxic, whereas six of eight isolates from two Dall sheep postinoculation were cytotoxic (Table 3). One of four *P. haemolytica* isolates obtained from preinoculation pharyngeal swabs of two bighorn sheep was cytotoxic; no samples were collected after inoculation from these bighorn sheep. All three preinoculation isolates from domestic sheep pharyngeal swabs were cytotoxic, as were three of five of the postinoculation isolates.

Dall sheep number 1 died within 24 hr of inoculation of the *P. haemolytica* A2 isolate. Of the two Dall sheep which were euthanized 52 hr after inoculation, Dall sheep number 2, which also received *P. haemolytica* A2, had increased respiration rates, and appeared depressed. Dall sheep number 3, which received a noncytotoxic

P. haemolytica T3,4,10 isolate, appeared clinically healthy. At necropsy, all Dall sheep were in good body condition with adequate amounts of body fat. Dall sheep numbers 1 and 2 had acute fibrinopurulent bronchopneumonia. Approximately 25 to 30% of the cranio-ventral portion of lungs from both animals were dark red and consolidated with small to moderate amounts of adherent fibrin.

Histologically, all cranio-ventral alveolar spaces contained cellular debris, edema fluid, erythrocytes, and basophilic bacterial colonies. In some areas, clusters of cells with elongated nuclei obliterated the alveolar lumen. Interlobular septa were often widened by fibrin, edema and necrotic cellular debris. Bronchial lymph nodes were characterized by diffuse congestion and edema. In one animal, the adrenal gland had vascular congestion throughout 80% of the parenchyma, and small aggregates of neutrophils were present in zona fasciculata. Central veins and sinusoids in liver of the same animal were congested. Sections of other tissues were considered normal. No gross or microscopic lesions of disease were detected in Dall sheep number 3.

All other animals remained clinically healthy during the experiment and were not euthanized. Isolates of *P. haemolytica* were recovered from all animals on the day of inoculation (Table 1) and from all but one animal on subsequent culture (Table 1). Viruses and lungworm larvae were not isolated from any animal.

DISCUSSION

Based on the cytotoxicity experiments, the preinoculation *P. haemolytica* isolates from pharyngeal swabs of healthy Dall sheep and bighorn sheep were primarily biotype T and predominantly non-cytotoxic (Table 1). In contrast, six of eight isolates obtained from preinoculation pharyngeal swabs of healthy domestic sheep were cytotoxic, including both A and T biotypes. Post-inoculation isolates obtained at necropsy from lung, lymph nodes and tonsils of the two pneumonic Dall sheep included biotype A, all of which were cytotoxic, and biotype T, all of which were non-cytotoxic (Table 1). Postinoculation isolates obtained from pharyngeal swabs of domestic sheep included both cytotoxic A and cytotoxic T biotypes. One unexpected finding in these experiments was the isolation of an untypeable biotype A cytotoxic *P. haemolytica* isolate from an apparently healthy bighorn sheep (number 1). Based on our experience, biotype A isolates are uncommon in healthy bighorn sheep, and this was the first cytotoxic isolate we have identified from a healthy bighorn sheep with no population history of respiratory disease (W. J. Foreyt, unpubl.). Because this result was inconsistent with data previously developed in our laboratory, we view this isolate as suspect and cannot rule out laboratory error. Further evaluation of isolates from healthy bighorn sheep will help to clarify the accuracy of this unusual finding.

Based on our results, Dall sheep are susceptible to pneumonia caused by the cytotoxic *P. haemolytica* A2 used for inoculation, but pneumonia did not develop in the one Dall sheep or two bighorn sheep inoculated with noncytotoxic *P. haemolytica* T3,4,10. Because the degree of neutrophil lysis of Dall sheep neutrophils in vitro was slightly greater than bighorn sheep neutrophils, we speculate that Dall sheep are at least as susceptible as bighorn sheep to pneumonia from the strains of *P. haemolytica* we tested from domestic sheep. We base these statements on results from these and previous experiments in which neutrophil sensitivity to cytotoxins (leukotoxins) from strains of *P. haemolytica* were correlated with host susceptibility to pneumonia (Silflow et al., 1993; Silflow and Foreyt, 1994; Foreyt et al., 1994; Sweeney et al., 1994). Bighorn sheep are susceptible to pneumonia caused by cytotoxic strains of *P. haemolytica* of domestic sheep origin (Silflow et al., 1993; Foreyt et al., 1994). In our experiment, one Dall sheep died within 24 hr of

intratracheal inoculation with a cytotoxic strain of *P. haemolytica* shown to be lethal in bighorn sheep. A second Dall sheep had developed signs of respiratory distress when it was euthanized 52 hr after inoculation.

To our knowledge, epizootics of pneumonia in wild Dall sheep caused by *P. haemolytica* have not been documented. However, results from our experiments provide significant evidence that exposure to at least one strain of *P. haemolytica* from domestic sheep may result in fatal pneumonia in Dall sheep. Dall sheep are often physically isolated from domestic sheep due to their remote natural habitats. However, with increased human habitation in areas where Dall sheep occur and in captive situations, the probability of fatal pneumonia from domestic sheep strains of *P. haemolytica* is greater. Based on the strong correlation between the in vitro and in vivo results obtained in these studies, we conclude that Dall sheep are highly susceptible to pneumonia caused by some strains of *P. haemolytica* of domestic sheep origin. One management strategy for preventing pneumonia in Dall sheep caused by strains of *P. haemolytica* carried by healthy domestic sheep is to prevent contact with domestic sheep.

Due to humane concerns and limited availability of surplus Dall sheep for experimental purposes, the sample size of live Dall sheep used in this study was limited to three animals. They were used in the inoculation experiment because they potentially had been exposed to a nonverified tuberculosis reactor and the United States Department of Agriculture (USDA) mandated their termination. Therefore we decided to use them in this experiment for the 52 hr before they were killed. We feel justified in their use because they were to be killed by the USDA, and we needed to evaluate the correlation between the data from the neutrophil cytotoxicity test and inoculation experiments. Because the in vivo and in vitro results were closely correlated, the neutrophil cytotoxicity test appears to be one in vitro method of evaluating data regarding susceptibility of specific ungulates to pneumonia caused by *P. haemolytica* (Silflow et al., 1994), and pathogenicity of *P. haemolytica* in wild sheep. Based on the data presented here and elsewhere, we believe in vitro neutrophil cytotoxicity assays provide important information for evaluating some aspects of the pathogenicity of strains of *P. haemolytica*. Further evaluation, refinement, and standardization of these techniques may provide justification to reduce the numbers of live wild sheep in experiments where potentially pathogenic *P. haemolytica* are tested in live animals to determine the importance of the host and disease agent interaction.

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