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EXPERIMENTAL CONTACT OF BIGHORN SHEEP (*OVIS CANADENSIS*) WITH HORSES AND CATTLE, AND COMPARISON OF NEUTROPHIL SENSITIVITY TO *PASTEURELLA HAEMOLYTICA* CYTOTOXINS

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ABSTRACT: Peripheral blood neutrophils from horses, cattle, and Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were evaluated for susceptibility to cytotoxin-dependent lysis of different biotypes and serotypes of *Pasteurella haemolytica* of domestic sheep, cattle, bighorn sheep, or mountain goat (*Oreamnos americana*) origin utilizing a cytotoxicity assay which measures the degree of bacteria cytotoxin-killing of neutrophils. All isolates of *P. haemolytica* (biotypes A and T) were noncytotoxic to horse neutrophils. Thirteen of 18 *P. haemolytica* biotype A isolates were cytotoxic (>50% neutrophil death in vitro) to bighorn sheep neutrophils, and four of 10 *P. haemolytica* biotype A isolates were cytotoxic to neutrophils of cattle; *P. haemolytica* biotype T (=*Pasteurella trehelosi*) isolates were noncytotoxic to neutrophils of bighorn sheep and cattle. When six bighorn sheep were pastured with three horses, only *P. haemolytica* biotype T isolates were recovered from the bighorn sheep throughout the study; *Pasteurella* spp. organisms were not isolated from the three horses. At initiation of a study where five bighorn sheep were pastured with three cattle, *P. haemolytica* biotype A, serotype 1, 2 was isolated from all three cattle, and only *P. haemolytica* biotype T isolates were recovered from the bighorn sheep. One bighorn sheep died in each of the horse and cattle copasturing experiments. *Pasteurella haemolytica* was not isolated from the bighorn sheep which died in the horse copasturing experiment. A noncytotoxic *P. haemolytica* biotype A, serotype 2 was isolated at necropsy from the bighorn which died in the cattle contact experiment. Based on these experiments, we believe bighorn sheep and horse association would not be detrimental to bighorns due to *P. haemolytica* induced pneumonia.

Key words: *Pasteurella haemolytica*, cytotoxin, neutrophil, bighorn sheep, domestic sheep, cattle, horses, experimental study.

INTRODUCTION

Bighorn sheep (*Ovis canadensis*) are highly susceptible to respiratory disease caused by the bacterium *Pasteurella haemolytica* (Silflow et al., 1991, 1993). Specific strains of *P. haemolytica* acquired from contact with domestic sheep have been implicated as a primary cause of pneumonia resulting in high mortality among bighorn sheep in both free-ranging and experimental conditions (Onderka and Wishart, 1984; Coggins, 1988; Foreyt, 1989; Foreyt et al., 1994). Deer (*Odocoileus* spp.) and elk (*Cervus elaphus*) neutrophils are not highly susceptible to the cytotoxin from *P. haemolytica* biotype A, serotype 2 from domestic sheep, but Dall sheep (*Ovis dalli dalli*), bighorn sheep, mountain goat (*Oreamnos americana*) and domestic sheep neutrophils are susceptible to cytotoxin-mediated injury (Silflow et al., 1994). *Pasteurella haemolytica* isolates cytotoxic to blood neutrophils in vitro and pathogenic to healthy bighorn sheep in vivo are predominantly biotype A, whereas biotype T isolates (also called *Pasteurella trehelosi*), are predominantly noncytotoxic to blood neutrophils and nonpathogenic to healthy bighorn sheep (Foreyt, 1994; Silflow et al., 1994).

Controlled experimental studies with bighorn sheep in contact with other ungulates have been conducted to determine if *P. haemolytica* can be transmitted to bighorn sheep from animals which free-ranging bighorn populations may potentially contact. Direct contact with domestic sheep or mouflon sheep (*Ovis musimon*) has resulted in mortality of bighorn sheep from acute bronchopneumonia (Foreyt, 1989, 1994). Bighorn sheep remained clinically healthy during contact exposure ex-

periments with llamas (*Lama glama*), domestic goats, mountain goats, cattle, deer and elk (Foreyt, 1992, 1994). However, in the cattle contact experiment, only *P. haemolytica* biotype T isolates were detected in the cattle throughout the study.

Cytotoxin production from *P. haemolytica* is a major virulence factor in the pathogenesis of pneumonia (Silflow et al., 1994). An in vitro assay has been developed for evaluating the cytotoxic potencies of different *P. haemolytica* isolates, and for evaluating neutrophil susceptibility to cytotoxin-mediated death (Silflow et al., 1993). In vitro, bighorn sheep blood neutrophils are highly vulnerable to lysis to cytotoxins produced by specific *P. haemolytica* isolates from domestic sheep (Silflow et al., 1993; Silflow and Foreyt, 1994). Distinguishing virulent from non-virulent *P. haemolytica* isolates based on cytotoxin potency would greatly aid wildlife managers to identify individuals among bighorn sheep and other animal species that harbor potentially virulent strains.

Our objectives were to determine the compatability of bighorn sheep with horses, and with cattle known to be carriers of *P. haemolytica* biotype A, and to evaluate and compare the susceptibility of horse, cattle and bighorn sheep neutrophils to cytotoxins from selected *P. haemolytica* isolates from domestic sheep, cattle, bighorn sheep or mountain goats.

MATERIALS AND METHODS

From 25 August 1994 to 12 June 1995, six Rocky Mountain bighorn sheep from the captive herd at Washington State University (WSU), Pullman, Washington (USA) and eight horses from the WSU equine facility were used as a source of blood neutrophils for the cytotoxicity assays. The bighorns ranged in age from 3 mo to 12 yr, and included five females and one male. The horses ranged in age from 4 yr to 22 yr, and consisted of two thoroughbreds, two quarterhorses, and one each of a Welsh-quarterhorse cross, Oldenburg, Clydesdale and Appaloosa. The horse and bighorn neutrophils were evaluated and compared for sensitivity to cytotoxins of nine *P. haemolytica* isolates from bighorn sheep, a domestic sheep, and a mountain goat. Seven of the isolates, identified by biotype A or T, followed by the serotype(s), were recovered from pharyngeal swabs of healthy bighorn sheep and included A untypeable (n = 4), A11 (n = 2), T3,4,10 (n = 1). The remaining isolates were an A6 isolate recovered from a mountain goat lung at necropsy, and an A2 isolate recovered from a pharyngeal swab of a healthy domestic sheep. The A2 isolate was cytotoxic to bighorn sheep neutrophils (Silflow and Foreyt, 1994.)

From 9 December 1994 to 21 April 1995, nine Rocky Mountain bighorn sheep from the captive herd at WSU and nine cattle, three from a ranch near Troy, Idaho (46°45′N, 116°45′W), and six from WSU, were used as sources for neutrophils. The bighorn sheep ranged in age from 6 mo to 12 yr, and included six females and three males. The cattle were Holstein steers between 6 and 18 mo. Neutrophils from the cattle were evaluated and compared to bighorn sheep neutrophils for sensitivity to cytotoxins of 12 *P. haemolytica* isolates from bighorn sheep, cattle, and a domestic sheep. Eight isolates were from bighorn sheep, including A2 (n = 4) and A untypeable (n = 1) isolated from the lungs of different bighorns at necropsy. The A2 isolates were recovered at necropsy from the lungs of bighorn sheep experimentally inoculated with a known cytotoxic strain of *P. haemolytica* A2 from domestic sheep. The other three isolates, A7,11 (n = 1), T4 (n = 1), and T3,4,10 (n = 1) were isolated from pharyngeal swabs of healthy bighorn sheep. Cattle isolates included A1,2 (n = 3) isolated from pharyngeal swabs from each of the clinically healthy cattle used in the copasturing study described herein. The final isolate tested was A2 (n = 1), isolated from a pharyngeal swab of a healthy domestic sheep and was the same domestic sheep cytotoxic isolate used in the horse and bighorn neutrophil comparison study.

For collection of blood, all animals were restrained physically, and approximately 40 ml of peripheral blood were collected from each animal by jugular venipuncture and placed in 8 ml of acid citrate dextrose solution. Following centrifugation at 850 × G for 20 min, the plasma and buffy coats were discarded. Hypotonic lysis of red blood cells was accomplished by the addition of 50 ml 0.001 M phosphate buffer solution for 50 sec followed by the addition of 25 ml 0.001 M phosphate buffer containing 2.7% sodium chloride. Following centrifugation at 600 × G for 15 min, the lysis and centrifugation steps were repeated, and the final neutrophil pellets were resuspended in Hanks balanced salt solution (HBSS) (Gibco Laboratories, Grand Island, New York, USA) containing 1% fetal bovine serum (FBS) (Hyclone Labo-

ratories, Logan, Utah, USA). The neutrophils 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.

The *P. haemolytica* biotype A isolates used in this study were selected because we suspected each isolate would be cytotoxic to bighorn sheep neutrophils in vitro. Cytotoxins were isolated from *P. haemolytica* culture supernatants using the method of Silflow et al. (1993). Individual *P. haemolytica* isolates were streaked onto 5% blood agar plates (Becton Dickinson Microbiological Systems, Cockeysville, Maryland, USA) and incubated for 18 to 24 hr at 37 C. A negative control bacteria, *Enterobacter cloacae* (American Type Culture Collection #35030, Rockville, Maryland), was handled identically. Several morphologically similar colonies were used to inoculate 115 ml of brain-heart infusion broth (Difco Laboratories, Detroit, Michigan, USA) which was placed in a shaking incubator at 37 C until cultures reached early logarithmic growth. To quantitate the number of bacteria, the optical densities 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 $\times$ *G* to a pellet, the supernatant discarded, and the pellet resuspended in 30 ml of RPMI-640 media (Gibco Laboratories) containing 7% FBS. Following incubation in a shaking incubator for 1 hr at 37 C, the bacteria again were centrifuged at 6,000 $\times$ *G* for 10 min, and the culture supernatants were removed and filter sterilized in a 0.45 µm filter (Sigma Chemical Company, St. Louis, Missouri, USA). Culture supernatants were dialyzed to exhaustion against distilled water and lyophilized.

Blood neutrophils from cattle, horses and bighorn sheep were evaluated for susceptibility to *P. haemolytica* cytotoxins by adding bacterial culture supernatants to the neutrophils in vitro. Cytotoxicity was determined at concentrations of supernatant of 150, 100, 50, 5 and 0.5 µg cytotoxin/50 µl HBSS containing 1% FBS (Silflow et al., 1993). All cytotoxin supernatants 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 2.5×10^5 neutrophils in 50 µl of HBSS containing 1% FBS to each well. Following 1 hr incubation at 37 C, 100 µl of lactate dehydrogenase (LDH) substrate was added to each well. The cytotoxicity of each supernatant concentration was quantitated by assessing the release of LDH from lysed neutrophils into the culture medium (Korzeniewski and Callewaert, 1983) using a 96-well plate reader coupled to an on-line computer (Silflow et al., 1993). 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 concentration lethal to 50% of the exposed cells (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.

Six Rocky Mountain bighorn sheep from a captive herd at WSU, including four females and two males ranging in age from 2 mo to 12 yr, were placed in a 0.5 ha pasture with three 3-yr-old Shetland cross horses from 14 July 1994 to 13 September 1994 (61 days).

Five Rocky Mountain bighorn sheep from a captive herd at WSU, including three females and two males ranging in age from 6 mo to 12 yr, were placed in a 0.5 ha pasture from 5 October 1994 to 8 December 1994 (64 days) with three 6-mo-old Holstein steers acquired from a ranch near Troy, Idaho, USA (46°45′N, 116°45′W).

The same pasture was used at different times for both experiments, and contained two covered shelters and various grasses. Alfalfa hay, alfalfa pellets, trace mineral salt and water were available at all times. With the exception of one old and thin bighorn ewe in the horse copasturing study, all animals were clinically healthy at the initiation of each experiment. All animals were observed twice daily for signs of clinical disease.

Pharyngeal swab samples for bacterial isolation were obtained from all animals at the beginning and end of each experiment. For bighorn sheep and cattle, a harp speculum was used to open the mouth and restrain the tongue, and a sterile rayon-tipped swab on plastic shaft (Spectrum Laboratories, Inc., Houston, Texas, USA) was grasped in a 23 cm hemostat and guided to the caudal pharynx. The swab was rubbed firmly against the tonsillar and pharyngeal region and removed. Bacterial swabs from horses were obtained by placing a 60 cm Accu-Culshure® swab (Accu-Med Corporation, Pleasantville, New York) into the nares and rubbing the pharyngeal region with the swab. All swabs were transported to the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington, and

streaked onto 5% blood agar plates (Benton Dickinson Microbiology Systems) within 2 hr of collection. Methods for isolation and identification of biotypes and serotypes of *P. haemolytica* isolates were those of Foreyt and Lagerquist (1994). Animals that died during the experiment were submitted to WADDL for necropsy evaluation. Samples of tissues, including lung, liver, spleen, tongue, and heart, were fixed in 10% buffered formalin, sectioned at 5 μm, and stained with hematoxylin and eosin. Lung and liver samples were evaluated for bacteria by the methods of Carter (1979) and Foreyt and Lagerquist (1994).

Statistical significance of the data was tested by 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) ($P < 0.05$).

RESULTS

All *P. haemolytica* isolates, biotypes A and T, were noncytotoxic to horse neutrophils (Table 1). Thirteen of 18 *P. haemolytica* biotype A isolates, comprising five serotypes, from domestic sheep, cattle, bighorn sheep and mountain goat, were cytotoxic to bighorn sheep neutrophils (Tables 1 and 2). Four of 10 *P. haemolytica* biotype A isolates, comprising three serotypes, from cattle and bighorn sheep were cytotoxic to cattle neutrophils (Table 2). All biotype T isolates were noncytotoxic to both bighorn sheep and cattle neutrophils.

Percent neutrophil death of bighorn sheep neutrophils for the cytotoxic isolates was significantly greater ($P < 0.05$) at all cytotoxin concentrations when compared to horse neutrophils (Table 1). When comparing percent neutrophil death between bighorn sheep and cattle from cytotoxic isolates, bighorn neutrophils were significantly more susceptible to cytotoxin-mediated cell death at cytotoxin concentrations of 100, 5, and 0.5 μg/50 μl ($P < 0.05$) (Table 2).

Pasteurella sp. was not isolated from the horses, and only *P. haemolytica* biotype T was detected in the bighorn sheep in the horse copasturing study. Isolates of *P. haemolytica* A1,2 were collected from all three cattle at initiation of the cattle and bighorn copasturing study. All three isolates were cytotoxic to bighorn sheep neutrophils in vitro; one of these three isolates was cytotoxic to cattle neutrophils (Table 2). Biotype T isolates were detected in all three cattle at the end of the experiment, in addition to A11 ($n = 1$) and A untypeable ($n = 1$). At initiation of the cattle copasturing study, biotype T was isolated from three of five bighorn sheep, and A untypeable in two bighorns; only biotype T isolates were detected in the bighorns at completion of the experiment.

One bighorn sheep died on experimental Day 22 of the horse and bighorn copasturing study. At necropsy, this bighorn sheep was in poor body condition. Three elongated incisors did not contact the maxillary dental pad, but protruded craniodorsally, and the second right maxillary molar was loose. The alveolar socket was sclerotic and thickened. Approximately 30% of the lungs contained multiple fibrinous plaques adherent to the pleura, and on cross section were mottled dark red to purple with irregular black patches rimmed with yellow fibrotic tissue. Histologically, lung tissue was characterized by diffuse intra-alveolar edema and multifocal hemorrhage. Both the interlobular septa and pleura were diffusely edematous, thickened and contained scattered foci of mixed inflammation. The pleural surface was covered with fibrin tags and degenerative inflammatory cells. *Pasteurella multocida* and *Streptococcus zooepidemicus* were isolated from the lung, but *P. haemolytica* was not isolated at necropsy. One bighorn sheep died on experimental Day 6 of the cattle and bighorn copasturing study. At necropsy, this bighorn sheep was in good body condition with adequate amounts of body fat. The lung lesions were consistent with severe bronchopneumonia. Histologically, alveoli and interstitium were filled with proteinaceous fluid and fibrin. *Pasteurella haemolytica* A2 was isolated from the lung. This isolate was tested in vitro with the cytotoxicity assay and was

TABLE 1. Cytotoxicity, expressed as percent neutrophil death, of *Pasteurella haemolytica* isolates to bighorn sheep and horse neutrophils.

Bacterial source	Biotype and serotype	Bighorn neutrophils (n = 6 bighorns) Cytotoxin concentration (μg/50 μl of supernatant)						Horse neutrophils (n = 8 horses) Cytotoxin concentration (μg/50 μl of supernatant)					
		150[a]	100	50	5	0.5	LC_{50}[a]	150	100	5	50	0.5	LC_{50}
Isolates cytotoxic to bighorn sheep neutrophils													
Domestic sheep	A2	54[b]	53	57	25	8	29	12	5	6	4	0	>150
Bighorn	A (unt)[c]	58	50	36	6	0	98	19	15	17	10	0	>150
Bighorn	A (unt)	36	47	69	23	8	19	6	3	5	2	0	>150
Mountain goat	A6	70	67	60	42	14	14	17	9	9	4	0	>150
Bighorn	A11	64	57	54	16	3	38	15	8	10	6	0	>150
Mean (SE)[d]		56 (6)[e]	55 (4)[e]	55 (5)[e]	22 (6)[e]	7 (2)[e]		14 (2)	8 (2)	9 (2)	5 (1)	0	
Noncytotoxic isolates													
Control	*Enterobacter* sp.	49	36	18	4	1	>150	15	8	8	4	0	>150
Bighorn	A (unt)	47	46	35	2	0	>150	16	14	17	11	0	>150
Bighorn	A (unt)	13	14	10	3	7	>150	10	8	7	2	0	>150
Bighorn	A11	31	33	29	28	0	>150	14	13	12	11	0	>150
Bighorn	T3, 4, 10	12	12	8	2	3	>150	11	7	7	3	0	>150
Mean (SE)		30 (8)	28 (7)[e]	20 (5)	8 (5)	2 (1)		13 (1)	10 (1)	10 (2)	6 (2)	0	

[a] 50% lethal concentration = the cytotoxin concentration (μg/50 μl of supernatant) resulting in 50% neutrophil death.
[b] Mean percentage of neutrophil death of six replicates at specified cytotoxin concentration.
[c] Unt, serotype untypeable due to autoagglutination.
[d] Standard error of the mean.
[e] Significantly different ($P < 0.05$) compared to horse neutrophils at specified cytotoxin concentration.

TABLE 2. Cytotoxicity, expressed as percent neutrophil death, of *Pasteurella haemolytica* isolates to bighorn sheep and cattle neutrophils.

Bacterial source	Biotype and serotype	Bighorn neutrophils (n = 9 bighorns) Cytotoxin concentration (μg/50 μl of supernatant)						Cattle neutrophils (n = 9 cattle) Cytotoxin concentration (μg/50 μl of supernatant)					
		150[a]	100	50	5	0.5	LC_{50}[a]	150	100	50	5	0.5	LC_{50}
Isolates cytotoxic to bighorn sheep neutrophils													
Domestic sheep	A2	47[b]	50	54	35	0	31	35	33	32	3	0	>150
Cattle	A1, 2	58	70	87	72	2	2	66	54	88	48	1	6
Cattle	A1, 2	67	61	49	13	1	51	49	35	20	4	0	>150
Cattle	A1, 2	61	55	49	7	1	55	40	29	14	3	0	>150
Bighorn	A2	62	68	54	48	3	11	61	53	49	4	0	59
Bighorn	A2	59	68	53	47	0	16	47	48	49	5	0	>150
Bighorn	A2	63	69	54	45	0	18	63	47	47	1	0	115
Bighorn	A (unt)[c]	62	66	52	49	1	11	52	51	51	3	0	47
Mean (SE)[d]		60 (2)	63 (3)[e]	57 (4)	40 (7)[e]	1 (0.4)[e]		52 (4)	44 (4)	44 (8)	9 (6)	0	
Noncytotoxic isolates													
Control	*Enterobacter* sp.	18	14	11	4	0	>150	16	11	6	4	0	>150
Bighorn	A2	27	21	13	0	0	>150	7	5	4	3	0	>150
Bighorn	A7, 11	2	20	14	11	2	>150	0	4	5	1	0	>150
Bighorn	T4	15	15	3	3	1	>150	7	3	2	2	0	>150
Bighorn	T3, 4, 10	10	7	5	2	0	>150	10	9	6	1	0	>150
Mean (SE)		14 (4)	15 (3)[e]	9 (2)	4 (2)	1 (0.4)		8 (3)	6 (2)	5 (1)	2 (1)	0	

[a] 50% lethal concentration = the cytotoxin concentration (μg/50 μl of supernatant) resulting in 50% neutrophil death.
[b] Mean percentage of neutrophil death of six replicates at specified cytotoxin concentration.
[c] Unt, serotype untypeable due to autoagglutination.
[d] Standard error of the mean.
[e] Significantly different ($P < 0.05$) compared to cattle neutrophils at specified cytotoxin concentration.

noncytotoxic to bighorn and cattle neutrophils. All other horses, cattle, and bighorn sheep remained clinically healthy during and after the copasturing experiments.

DISCUSSION

Based on the results from the in vitro cytotoxicity experiments, we believe bighorn sheep neutrophils are highly susceptible to cytotoxin-mediated cell death from the specific *P. haemolytica* biotype A isolates tested when compared to cattle and horses. The biotype T isolates from bighorn sheep were noncytotoxic to bighorn sheep, cattle, and horse neutrophils. These results are consistent with previous observations that *P. haemolytica* biotype A isolates are often cytotoxic to bighorn sheep blood neutrophils, but biotype T isolates are predominantly noncytotoxic (Foreyt, 1994; Silflow et al., 1994). Four of the eight isolates cytotoxic to bighorn sheep neutrophils in the cattle and bighorn neutrophil comparison study were of bighorn sheep origin, all recovered at necropsy from the lungs of bighorn sheep experimentally inoculated with a known cytotoxic *P. haemolytica* A2 from domestic sheep (Table 2). Of the three isolates of bighorn sheep origin cytotoxic to bighorn sheep neutrophils in the horse and bighorn neutrophil comparison study, two were recovered from pharyngeal swabs of healthy bighorn sheep from a captive herd at WSU, and one was from a pharyngeal swab of a healthy free-ranging bighorn sheep (Table 1). Four isolates of *P. haemolytica* biotype A were cytotoxic to cattle neutrophils, with three of these isolates recovered at necropsy from the lungs of bighorn sheep experimentally inoculated with *P. haemolytica* A2 of domestic sheep origin (Table 2). The role of *P. haemolytica* in causing pneumonia and death in cattle is well documented (Whiteley et al., 1992).

Five of 18 *P. haemolytica* biotype A isolates were noncytotoxic to bighorn sheep neutrophils, including A untypeable (n = 2), A2 (n = 1), A7,11 (n = 1) and A11 (n = 1) (Tables 1 and 2). All five isolates were from bighorn sheep, including four from pharyngeal swabs of clinically healthy bighorns. Based on our experience, it is very uncommon to isolate *P. haemolytica* biotype A from healthy bighorn sheep. Noncytotoxic A11 and A untypeable *P. haemolytica* isolates have been reported previously (Silflow et al., 1994).

Pasteurella haemolytica A2 was recovered at necropsy from the lung of the bighorn which died on Day 6 of the cattle copasturing experiment, and this isolate was noncytotoxic to bighorn and cattle neutrophils in vitro (Table 2). We expected that this isolate would be cytotoxic to bighorn sheep neutrophils in vitro, however, it is possible that this isolate was not involved in the fatal pneumonia. *Pasteurella* sp. was not isolated from a pharyngeal swab from this animal at initiation of the experiment. Silflow et al. (1993) acknowledged that different *P. haemolytica* isolates have varying degrees of cytotoxic potencies. Genomic fingerprinting and classification by ribotype may be necessary to more specifically identify the varying degrees of cytotoxic potencies among similar isolates than the traditional biotype/serotype classification of bacteria. Different ribotypes are present within the same serotype (Foreyt et al., 1994) and may account for the different cytotoxic potencies.

In a previous study, copasturing bighorn sheep and cattle did not result in respiratory disease in the bighorn sheep, but *P. haemolytica* biotype A isolates were not detected in the cattle throughout the experiment (Foreyt, 1994). *Pasteurella haemolytica* A2 is routinely isolated from the nasal passages of clinically healthy cattle, whereas *P. haemolytica* A1 is implicated commonly in the shipping fever complex (Whiteley et al., 1992). Perhaps the bighorn which died contracted the *P. haemolytica* A2 from the cattle or from another unidentified source. *Pasteurella haemolytica* A2 from clinically healthy domestic sheep is usually lethal when contracted by bighorn sheep (Foreyt, 1994), but a similar relationship between

cattle and bighorn sheep has not been reported. Although some strains of *P. haemolytica* carried by cattle are potentially lethal to bighorn sheep, the social interactive behavior between cattle and bighorn sheep is less compatible when compared to the domestic sheep and bighorn sheep interaction, and the nose to nose contact required for transmission of *P. haemolytica* is less likely to occur between cattle and bighorn sheep.

The bighorn which died in the horse copasturing study was greater than 12 yr old, and was in poor body condition at the onset of the study. The poor body condition was attributed to malnutrition due to decreased foraging capability caused by poor dentition. Both *P. multocida* and *Streptococcus zooepidemicus* were isolated from the lung at necropsy, but *P. haemolytica* was not recovered. Because of the lung lesions present, *P. multocida* may have contributed to the death. It is not likely that the bighorn sheep death was related to the association with horses. Prevalence of *Pasteurella* spp. from the respiratory tract of horses is generally low (Sweeney et al., 1985; Kester et al., 1993). In a review of the literature, we failed to find a single report of *P. haemolytica* isolated from the respiratory tract of over 3,000 healthy and pneumonic horses (Kester et al., 1993; and others).

Based on the in vitro results in which bighorn neutrophils were susceptible to *P. haemolytica* A1,2 isolates from cattle, and the death of one bighorn in the cattle compatibility study in which *P. haemolytica* A2 was recovered at necropsy, we recommend that further studies be conducted to determine the compatibility of cattle and bighorn sheep. Based on our preliminary studies with a limited number of horses, we believe that horses and bighorn sheep are capable of existing together without serious risk of *P. haemolytica* induced pneumonia to the bighorn sheep.

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