

Susceptibility of Rocky Mountain Bighorn Sheep and Domestic Sheep to Pneumonia Induced by Bighorn and Domestic Livestock Strains of *Pasteurella haemolytica*

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ABSTRACT

Bighorn sheep were inoculated intratracheally with suspensions of nonhemolytic *Pasteurella haemolytica* biotype T (10^{12} organisms) unique to wild bighorns, with beta-hemolytic *P. haemolytica* biotype T (10^{12} organisms) isolated from clinically normal domestic sheep or intradermally with half a dose of a cattle vaccine containing *P. haemolytica* biotype A (10^5 organisms). The bighorn strain caused lobar necrotizing bronchopneumonia whereas both domestic livestock strains precipitated fatal septicemia and fibrinous bronchopneumonia. The serotypes given were T3, T4, T15 and A1 and these were recovered from lung lesions and other organs.

In three trials, domestic sheep were inoculated intratracheally with suspensions of bighorn sheep pneumonic lungs, and two concentrations of the *P. haemolytica* bighorn strain (10^4 and 10^{12} organisms). One of these sheep was inoculated intrabronchially. The domestic sheep experienced a transient fever and elevated white blood cell counts. After six days, none of the sheep had lung lesions and inoculated organisms could not be recovered. It is suggested that bighorn sheep are very susceptible to *P. haemolytica* from domestic livestock and should not be allowed in contact with sheep or cattle.

RÉSUMÉ

Cette expérience consistait à introduire dans la trachée de mouflons des Rocheuses 10^{12} bactéries du biotype T de *Pasteurella haemolytica* non hémol-

ytique, propre à ces mouflons, ou la même quantité du biotype T de *P. haemolytica* bêta hémolytique, isolé de moutons domestiques sains. Elle visait aussi à leur administrer, par la voie intradermique, la moitié d'une dose de vaccin destiné aux bovins, laquelle contenait 10^5 bactéries du biotype A de *P. haemolytica*. La souche propre aux mouflons causa une broncho-pneumonie lobaire nécrotique, alors que les deux souches des ruminants domestiques précipitèrent une septicémie fatale et une broncho-pneumonie fibrineuse. Les sérotypes utilisés étaient les suivants: T3, T4, T15 et A1; on les recouvra des lésions pulmonaires et des autres organes.

Dans trois expériences, on introduisit dans le trachée de moutons domestiques des suspensions de tissu pulmonaire lésé des mouflons, ainsi que 10^4 et 10^{12} bactéries de la souche de *P. haemolytica* qui leur est propre. Un de ces moutons reçut l'inoculum dans les bronchioles. Les moutons manifestèrent une hyperthermie et une leucocytose transitoires. Au bout de six jours, aucun d'eux n'affichait de lésions pulmonaires et on ne réussit pas à recouvrer les bactéries qu'on leur avait inoculées. Il semble donc que les mouflons des Rocheuses sont très susceptibles aux *P. haemolytica* des animaux domestiques et qu'on ne devrait pas les laisser venir en contact avec les moutons et les bovins.

INTRODUCTION

Pasteurella pneumonia has been recognized for many decades as an important component of die-offs in bighorn sheep (*Ovis canadensis cana-*

denis) (1-5). In some cases, *Pasteurella* spp. was the only infectious agent isolated (6,7) but in most investigations other bacteria, lungworms and viruses were also incriminated in the respiratory disease complex (3,6,8,9-11). Two die-offs were associated with proximity to domestic livestock or the use of bighorn habitat by domestic sheep (12) causing friction between wildlife managers and livestock owners over pasture use. A major die-off caused by pneumonia in bighorn sheep of all ages occurred in southern Alberta in 1981-82 (7). *Pasteurella haemolytica* biotype T, nonhemolytic variant, was isolated from infected lungs, tonsils, bronchial nodes and nasal passages. There was no apparent correlation with extensiveness of lungworm infection, and virus, *Mycoplasma* spp. and *Chlamydia* spp. isolation attempts were negative. Bacterial cultures from tonsils of 61 healthy bighorn sheep throughout Alberta showed 25% to be carriers of this particular *Pasteurella* strain (Onderka, unpublished data) whereas cultures of nasal swabs from 239 bighorns were negative for *Pasteurella* spp. (7). In this study, we investigated the pathogenicity of the bighorn sheep *Pasteurella* isolate in bighorns and domestic sheep and examined the susceptibility of bighorn sheep to *P. haemolytica* isolated from domestic livestock.

MATERIALS AND METHODS

ANIMALS

Bighorn sheep were collected from several locations in Alberta by the use of corral traps baited with salt, or by the

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administration of 150 mg xylazine (Haver-Lockhart Laboratories, Shawnee, Kansas) delivered by a projectile dart (Cap-Chur gun, Palmer Chemical and Equipment Co. Ltd., Douglasville, Georgia). The guidelines of the Canadian Council on Animal Care for wild vertebrates were observed. The sheep were transported in individual crates under xylazine sedation (4 mg/kg intramuscularly). They were housed individually in quiet, well lit indoor stalls measuring 300 x 140 cm and 180 cm high at 15-20°C. Due to the intense flight reaction of wild sheep, the stalls consisted of three solid walls, a gate of dense wire mesh and a ceiling of chainlink fencing wire. During the adaptation period of about one week, the ceiling was covered with plywood and the gate darkened with black, roll down plastic. Each animal readily ate good quality, free-choice hay supplemented daily with 250 g of alfalfa pellets (Masterfeeds, Edmonton, Alberta) and 2 g cobalt-iodized stock salt (Masterfeeds) sprinkled on the hay every other day. Water was provided in a secured bucket. The floor was covered with straw. Regular cleaning was done while the sheep were blindfolded, which kept them calm and made them easy to handle to obtain rectal temperature, blood samples, nasal swabs and fecal samples (for parasitology). Hooves were trimmed as necessary. Sheep were euthanized using an overdose of pentobarbital (M.T.C. Pharmaceuticals, Mississauga, Ontario).

INOCULA

Pasteurella haemolytica biotype T cultures were obtained from pneumonic lung tissues during the 1981-82 die-off and subsequent mortalities due to pneumonia in bighorn sheep and from tonsils of domestic sheep during a slaughterhouse survey in which 40 animals from nine areas throughout Alberta were sampled. Specimens were plated directly onto blood agar (BAP; tryptic soy agar base [Difco, Detroit, Michigan] containing 5% sheep blood), MacConkey agar (MAC; [Difco]) and chocolate agar (Proteose #3 agar base [Difco] containing 2% hemoglobin solution [Difco] and supplement B [Difco]). Identification of *P. haemolytica* was

made according to recognized characteristics (13,14). Further differentiation between biotype A and biotype T was done by the use of specific sugar reactions (13,14). *Pasteurella haemolytica* biotype T isolated from bighorn sheep was further characterized by its total lack of hemolysis which remained throughout multiple subcultures. Some salient biochemical features, based on 60 isolates, are presented in Table I. This strain will be referred to as "*P. haemolytica* biotype T, BHS strain". *Pasteurella haemolytica* biotype A originally isolated from cattle, was contained in a commercial vaccine (Precon PH, Vetrepharm, London, Ontario). All isolates, except the vaccine, were suspended in sheep blood and stored at -70°C where they remained viable after four years. Isolates used for this study were recultured, from storage, onto blood agar plates and checked for purity. Selected colonies were picked, transferred to chocolate agar slants and sent to Dr. W. Donachie at the Moredun Research Institute, Edinburgh, Scotland who kindly provided the serotyping and confirmed the biotypes. Bacterial suspensions (antigen preparation) were prepared from overnight brain-heart infusion broth cultures (BHI; Difco), and harvested by centrifugation, washed three times with sterile phosphate buffered saline (PBS; Difco) and resuspended in sterile PBS. Viability counts were determined by a plate count method using consecutive decimal dilutions

plated onto BAP. Counts of viable organisms per mL of bacterial suspension were determined by multiplying an average of total colonies per plate by the amount sampled and by the reciprocal of the dilution used.

VIRUS ISOLATION

Lung tissue was ground in phosphate-buffered saline, centrifuged, and the supernatant inoculated into 12th passage bighorn sheep fetal lung tissue culture cells grown in tissue culture flasks containing Eagle's minimum essential medium with 10% fetal bovine serum (Gibco Laboratories, Live Technologies Inc., Grand Island, New York). Cultures were incubated at 37°C and examined daily for one week for cytopathic effects. The flasks were then frozen, thawed and reinoculated for a further week of incubation.

EXPERIMENTS

A: *Pasteurella haemolytica* BHS strain inoculated into bighorn sheep. One bighorn lamb was inoculated intratracheally with 2 mL sterile PBS containing 3.2×10^{12} organisms of a composite of *P. haemolytica* biotype T, BHS strain, isolated from three pneumonic lungs collected during the bighorn sheep die-off. A second bighorn lamb was inoculated with sterile PBS and served as a control.

B. *Pasteurella haemolytica* from domestic sheep inoculated into bighorn sheep. A composite of betahemolytic

TABLE I. Some Morphological and Biochemical Characteristics of *Pasteurella haemolytica* Biotype T, Nonhemolytic Strain from Bighorn Sheep

Characteristic		% Positive ^a
Morphology on BAP	nonhemolytic greys	100
Growth on MAC	+	100
Gram stain	gram-negative, rod shaped bacilli, some pleomorphism; bipolar staining	100
Catalase	—	0
Oxidase	+(weak reactions common)	100
<i>Acid from:</i>		
Lactose	—	0
Mannitol	+	100
Trehalose	+	100
Xylose	—	0
Arabinose	—	0
Salicin	+	100
Esculin hydrolysis	+	100
Indole ^b	—	0
Urea	—	0

^aBased on 60 isolates; +: positive in 7-10 days; —: negative reaction in 14 days

^bIndole reaction read within 48 h

P. haemolytica biotype T isolated from tonsils of three healthy slaughtered domestic sheep was given intratracheally to two yearling bighorn sheep each at a concentration of 2×10^{12} organisms in 2 mL sterile PBS.

C. Cattle vaccine inoculated into bighorn sheep. Eight captive bighorn sheep for public display (adults and young of the year) were inoculated intradermally with 5×10^5 organisms of *P. haemolytica* biotype A contained in 0.25 mL of the commercial vaccine.

D. *Pasteurella haemolytica* BHS strain inoculated into domestic sheep. Nine 2 yr old Suffolk sheep were used to test the pathogenicity of BHS isolates for domestic sheep in three separate trials. In trial I, two sheep were inoculated intratracheally with 2 mL homogenate of pneumonic bighorn sheep lung filtered through a sterile, coarse cloth. One sheep was inoculated intratracheally with 1×10^4 viable organisms of *P. haemolytica* biotype T, BHS strain, while a fourth sheep served as a sham inoculated control. In trial II, two sheep were inoculated intratracheally with 1.6×10^{12} and 3.2×10^{12} organisms of *P. haemolytica* biotype T, BHS strain, in 1 mL and 2 mL sterile PBS respectively, using the same combined cultures as in experiment A. A third sheep served as control. In trial III, one sheep was inoculated intratracheally with 1×10^{12} organisms of the composite BHS culture in 2 mL sterile PBS while a second sheep received 5×10^{12} organisms in 10 mL sterile PBS given intrabronchially using a 56 cm long 3.5 FR polypropylene catheter (Monojet, St. Louis, Missouri) introduced in mid-trachea through a 14 G hypodermic needle.

RESULTS

Ten *Pasteurella* BHS strain isolates were serotyped as T4 (five), T15 (four) and T3 (one), indicating no correlation between the common lack of hemolysis and serotypes.

A: *Pasteurella haemolytica* BHS strain inoculated into bighorn sheep. One yearling male was inoculated with

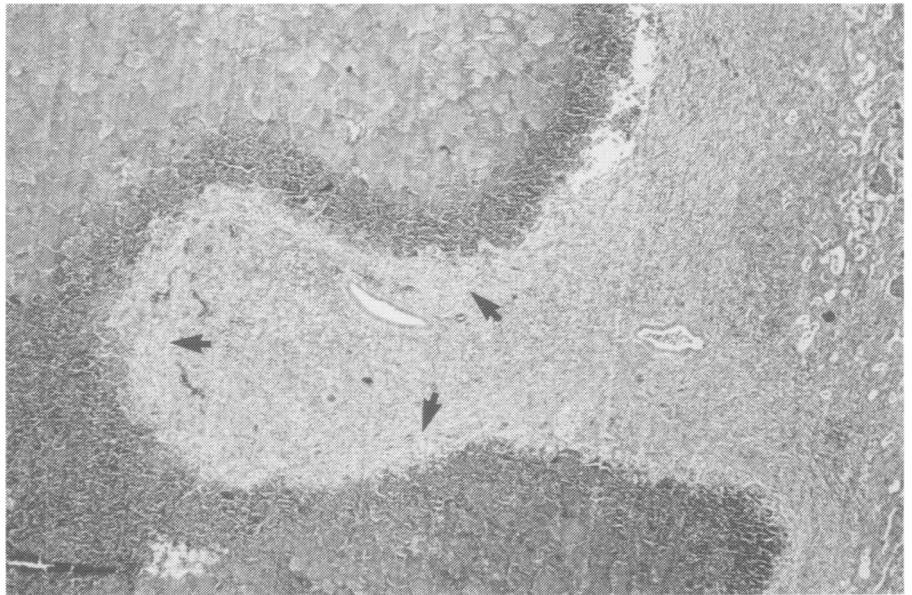


Fig. 1. Lung from bighorn sheep inoculated intratracheally with 3.2×10^{12} viable organisms of *P. haemolytica* biotype T, BHS strain and killed ten days postinoculation. There is extensive necrosis, delineated by degenerated inflammatory cells, surrounded by fibrosis (arrows). H & E X23.

serotypes T4 and T3. The rectal temperature of the inoculated sheep rose from 39°C to 40°C within 26 h postinoculation. It remained elevated for two days and then returned to a constant $38\text{--}38.5^\circ\text{C}$. No temperature rise occurred in the control sheep. Total white blood cells increased from a preinfection level of 8,800 to 10,725 per μL in 48 h with an increase in neutrophils from 55 to 65%. By day 5 the values returned to normal. The control sheep maintained a total white blood cell count of 8,800–8,900 per μL . Nasal swabs from the experimental and the control sheep taken three and eight days postinoculation showed mixed, scant growth of *Bacillus* spp., *Staphylococci* spp., *Streptococci* spp. and beta-hemolytic *P. haemolytica* biotype A. No clinical signs were observed and the animals were euthanized ten days postinoculation. Necropsy of the inoculated bighorn sheep revealed an enlarged, right cranial lung lobe, adhered rather firmly to the thoracic wall. Cut sections showed multiple areas of peribronchial necrosis and well demarcated dark red, firm pneumonic tissue. Bronchial lymph nodes and tonsils were enlarged. There was a light to moderate *Protostrongylus stilesi* infection in the posterior caudal lobes. *Protostrongylus rushi* nema-

todes were found in the mainstem bronchi. Histological examination showed fairly extensive areas of coagulation necrosis surrounded by degenerated inflammatory cells and fibrous tissue (Fig. 1). Alveoli were filled with fibrin, neutrophils and cell debris. Interlobular septa were thickened by edema and organizing fibrin. Adjacent parenchyma was congested, alveoli were collapsed and there was some hemorrhage. Other areas of the lungs were normal except where the parasite infection elicited lymphocyte infiltration mixed with a few neutrophils associated with embryonated eggs. Adult nematodes of *P. rushi* in the larger bronchioles caused no host reaction. The control sheep had no visible gross or microscopic lesions except for those associated with a mild lungworm infection. Cultures were negative except for nasal swabs as described above. Bacterial cultures of lung and tonsils from the experimental sheep gave pure growth of the nonhemolytic *P. haemolytica* BHS strain yielding serotype T4 and T15. Bronchial nodes were negative. Nasal swabs still showed a mixed culture including beta-hemolytic *P. haemolytica* biotype A. Isolation attempts for viruses, *Mycoplasma* spp. and *Chlamydia* spp. were negative.

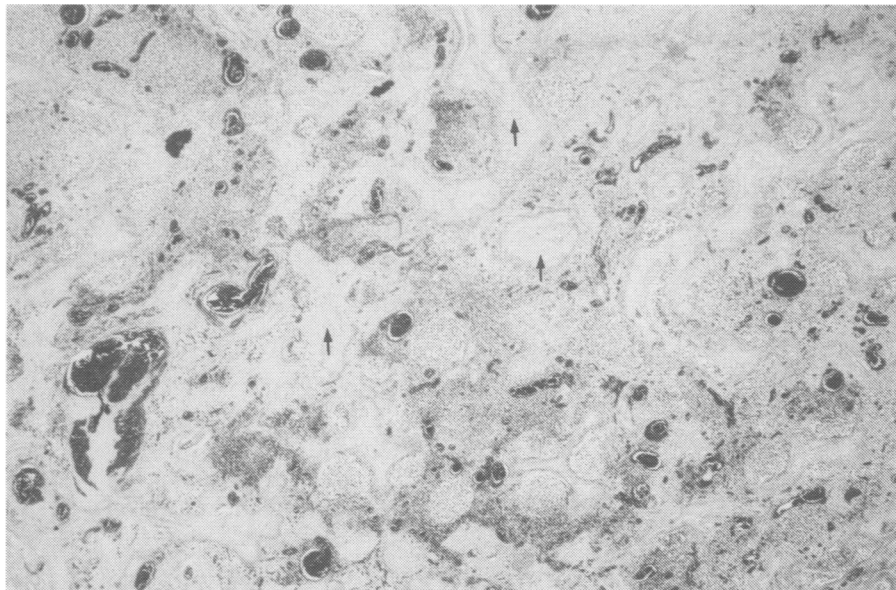


Fig. 2. Lung from bighorn sheep inoculated intratracheally with 2×10^{12} organisms of *P. haemolytica* biotype T from tonsils of normal domestic sheep. This sheep died 42 h postinoculation. There are bands and islets of coagulation necrosis (arrows), diffuse alveolitis and congestion with some hemorrhage. H & E X23.

B: *Pasteurella hemolytica* from domestic sheep inoculated into bighorn sheep. Two 1 yr old bighorn sheep were inoculated intratracheally with a composite of isolates from three different domestic sheep. Only one of these was serotyped and identified as T15. Ten hours postinoculation, sheep no. 1 showed labored breathing and appeared lethargic. It died approximately 16 h postinoculation. Sheep no. 2 showed similar respiratory distress after 24 h. It had an elevated rectal temperature of 41°C. The animal remained recumbent and did not eat. It died 42 h postinoculation. On necropsy, sheep no. 1 had extensive subcutaneous hemorrhages over the neck, thighs and abdomen. The tracheal mucosa was congested and tonsils were slightly enlarged. The thoracic cavity contained 200 mL of clear, straw colored fluid and petechial hemorrhages were seen on the epicardium. Red areas of consolidation were seen in the right and left cranial lung lobes and in the ventral middle lobe. A light lungworm infection was noted in the posterior caudal lobes. The abomasal mucosa was severely congested and had multiple areas of hemorrhage. Microscopic examination confirmed the congestion and hemorrhage described above. Hem siderin accumulated in the spleen

while multiple foci of bacterial growth was present in spleen and liver. There was hemorrhage in the mid-cortical regions of the kidneys. Beta-hemolytic *P. haemolytica* biotype T, serotype 4 was isolated from tonsils, lungs and kidneys. *Corynebacterium pyogenes* was also isolated from the tonsils.

Sheep no. 2 had much stable froth in the trachea and bronchi. The right cranial and middle lung lobes were enlarged, dark red and firm. The left cranial lobe had areas of congestion. Both cranial lobes had fibrinous adhesions to the thoracic wall and the pericardial sac. Tonsils were slightly enlarged while bronchial lymph nodes were moderately enlarged. There was severe petechiation of the pericardial sac and the epicardium. Abdominal lymph nodes were markedly enlarged. Histologically there were hemorrhage and edema fluid accumulating in alveoli, and oat-shaped macrophages filling many alveoli. Areas of coagulation necrosis were present. Interlobular septa were thickened by edema and fibrin (Fig. 2). Bronchial lymph nodes were edematous, had areas of necrosis and hemorrhage, and foci of bacteria. Lymphocyte hyperplasia and hemorrhage were seen in abdominal lymph nodes. Adrenal glands were congested and had cortical hemorrhage; liver and kidneys were also congested.

Bacterial cultures gave pure growth of beta-hemolytic *P. haemolytica* biotype T, serotype 15 from lungs, tonsils, spleen, bronchial and abdominal lymph nodes and kidneys.

C. Cattle vaccine inoculated into bighorn sheep. A half dose of cattle vaccine containing *P. haemolytica* biotype A, serotype 1 was administered intradermally to eight bighorn sheep ranging in age from 3 wk to 4 yr. One adult female and three lambs died three days postvaccination. A second adult female died on day 4 and all sheep were submitted to our veterinary laboratory. The other three sheep survived under antibiotic treatment. The lesions on days 3 and 4 were those of acute, fibrinous bronchopneumonia involving about one-half of the lungs with fibrinous adhesions of lung lobes to the pericardium. The epicardium had petechial hemorrhages. In one of the lambs both lungs were red and firm and much stable froth was in the trachea. Microscopic examination of the lungs of all sheep showed alveolar hemorrhage and edema with neutrophil and oat-shaped cell infiltrations. Interlobular septa were thickened by fibrinous exudate (Fig. 3), and there were occasional venous thrombi. Lungworm infection was minor or absent. Bacterial cultures gave pure growths of *P. haemolytica* biotype A, serotype 1 from all lungs. From one ewe, *P. haemolytica* biotype T, BHS strain was cultured from the tonsils.

D. *Pasteurella hemolytica* BHS strain inoculated into domestic sheep. All seven sheep inoculated with cultures had a transient 1°C elevation of rectal temperature about 2-4 h postinoculation. There was also a slight elevation of total white blood cells with an increase in neutrophils. The levels returned to normal after three days. Nasal swabs sporadically yielded *P. haemolytica* biotype A. The animals were euthanized six days postinoculation. There were no gross lesions except for an enlarged retropharyngeal lymph node in one sheep of trial I and a small focus of red consolidation in the anterior lung of one sheep in trial III. Microscopic examination showed no significant lesions. Bacterial cultures of lungs and bronchial

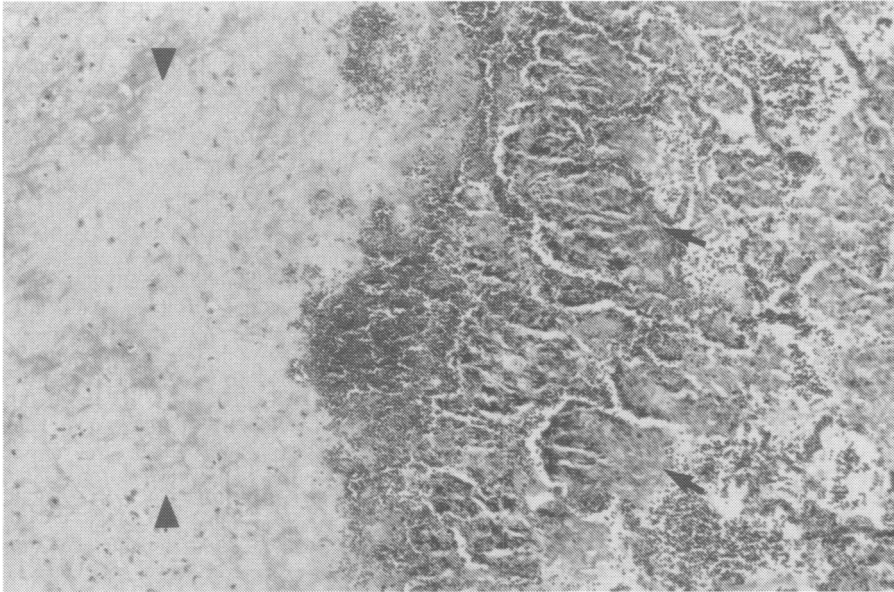


Fig. 3. Lung from bighorn ewe inoculated intradermally with 5×10^5 organisms of *P. haemolytica* biotype A, serotype 1 contained in a cattle strain vaccine. This ewe died three days postinoculation. Alveoli are infiltrated with spindle shaped inflammatory cells (arrows). There is hemorrhage into alveoli and interlobular septum which is greatly distended with fibrinous exudate (arrow heads). H & E X113.

nodes were negative. *Pasteurella haemolytica* biotype A was isolated from tonsils of three sheep.

DISCUSSION

Pasteurella pneumonia in sheep and cattle has been the subject of extensive research to ascertain the pathogenicity of this opportunistic agent and the pathogenesis of lesions it produces (15-17). Experimental reproduction of the disease was inconsistent, often requiring predisposing virus or *Mycoplasma* spp. infections (18-20). Successful infection usually required large numbers of bacteria to be introduced into the lung (21,22) which may not mimic the circumstances of a natural infection (23). Conversely, it is not certain how many bacteria are transmitted through aerosol from the nose and mouth of a coughing, pneumonic sheep. Nasal bacterial flora certainly changes dramatically during stress or pasteurellosis (24-26). A similar dilemma exists in the investigation of pneumonia in bighorn sheep in which predisposing factors include lungworm infections (1,8) and serological but rarely cultural evidence of virus infection (9-11). There are, however, reports of bighorn sheep

suffering from *Pasteurella* pneumonia without involvement of other agents (1,6,7). The present study was not designed to investigate the pathogenesis of *P. haemolytica* pneumonia as it occurs in the wild but to demonstrate the susceptibility of bighorn sheep to various *Pasteurella* strains under controlled conditions.

The results showed that *P. haemolytica* BHS strain is capable of producing pneumonic lesions in bighorn sheep without the known presence of other agents. The recovery of the same serotype supported the inference that the lung lesions were indeed caused by the inoculum used. It must be pointed out, however, that a variety of serotypes may be present on a culture plate (27) and not all may be picked for serotyping. Thus some serotypes may not be described in the inoculum but are identified in cultures from the lesions and vice versa. The cultural characteristic of nonhemolysis remained. The microscopic appearance suggested a similar time sequence in the development of lesions as described for cattle (28). By comparison, *Pasteurella* spp. isolated from tonsils of clinically normal domestic sheep inoculated at a similar dose resulted in a severe septicemia quickly causing death in the bighorn sheep. It

is of interest that one of the bighorn sheep had intra-alveolar oat-shaped inflammatory cells which are characteristic of *P. haemolytica* infection in cattle and sheep (29) but are never seen in pasteurellosis of bighorn sheep caused by the nonhemolytic BHS strain. The cattle vaccine was inoculated at half the recommended cattle dose, as is used to vaccinate domestic sheep against pasteurellosis. In this case however, the relatively low dose given intradermally resulted in fatal septicemia with primary involvement of the lungs. There was a difference between the domestic *P. haemolytica* biotype A and biotype T strains in that biotype T caused widespread septicemia while biotype A involved mainly the lungs. This difference was also observed in domestic sheep (30) but not in cattle. Again, recovery of the same serotype from the lesions suggested the vaccine strain to be the pathogen.

These results showed that bighorn sheep are extremely susceptible to domestic livestock strains of *P. haemolytica* and should not be allowed in contact with sheep or cattle. This was further substantiated experimentally by the transmission of *Pasteurella* spp. from clinically normal domestic sheep to captive wild bighorn sheep in close contact, resulting in severe pneumonia (31).

Repeated attempts to induce pneumonia in domestic sheep using similar challenge doses of the *Pasteurella* BHS strain failed to cause any lesions. Furthermore, it was never possible to recover the inoculated *Pasteurella* strain from the lungs or tonsils. The marked difference in pulmonary response to *Pasteurella* challenge between the two sheep species will be further investigated.

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