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FATAL PNEUMONIA FOLLOWING INOCULATION OF HEALTHY BIGHORN SHEEP WITH *PASTEURELLA HAEMOLYTICA* FROM HEALTHY DOMESTIC SHEEP

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ABSTRACT: In a series of three experiments, isolates of *Pasteurella haemolytica* biotype A, serotype 2, ribotype reference WSU-1, from healthy domestic sheep, were inoculated intratracheally into eight bighorn sheep (*Ovis canadensis canadensis*) and seven domestic sheep with doses of bacteria ranging from 5.3×10^6 to 8.6×10^{11} colony forming units. Seven of eight inoculated bighorn sheep died from acute pneumonia within 48 hr of inoculation, whereas all seven domestic sheep inoculated with comparable or greater doses of bacteria remained healthy. One contact control bighorn sheep also died 6 days after its penmates received *P. haemolytica*. Three other noncontact control bighorn sheep remained healthy during the experiments. *Pasteurella haemolytica* biotype A, serotype 2, ribotype reference WSU-1 in the inocula was recovered from one or more tissues from all bighorns that died; whereas, it was not detected in any bighorn sheep before inoculation. Three different ribotypes of *P. haemolytica* A2 were recovered from bighorn sheep; however, only the ribotype reference WSU-1 in the domestic sheep-origin inoculum was recovered from all dead bighorn sheep, and was not recovered from bighorn sheep that survived the experiments. Thus, a relatively nonpathogenic and common isolate of *P. haemolytica* from healthy domestic sheep was lethal in bighorn sheep under experimental conditions.

Key words: *Pasteurella haemolytica*, bighorn sheep, pneumonia, *Ovis canadensis*, domestic sheep, experimental infection, transmission, ribotyping.

INTRODUCTION

Respiratory disease is a major mortality factor in bighorn sheep (*Ovis canadensis*) populations and has had a major impact on their population dynamics by severely limiting population growth. Lungworms, viruses, bacteria, especially *Pasteurella haemolytica*, and stress associated with a variety of factors, frequently are identified as components of pneumonia epizootics in bighorn sheep (Spraker et al., 1984; Foreyt, 1989; Miller et al., 1991). Recently, domestic sheep also have been implicated as important in some bighorn sheep pneumonia epizootics, because close contact between the species under experimental or field conditions has resulted in bighorn sheep pneumonia with high mortality (Coggins, 1988; Foreyt, 1989, 1992). Although *P. haemolytica* can be isolated routinely from healthy bighorn sheep (Dunbar et al., 1990a, b; Wild and Miller, 1991), it has been speculated that some strains of *P. haemolytica* from healthy domestic sheep are highly pathogenic in healthy bighorn sheep (Onderka and Wishart, 1988; Foreyt, 1989). However, the mechanisms regulating this disease interaction are not fully understood. Determining the role of domestic sheep in the epizootiology of bighorn sheep pneumonia is important. State and federal land use decisions regarding domestic livestock grazing are often based in part on predicted impact of livestock grazing on wildlife health and survival. Contact with domestic sheep can potentially compromise bighorn sheep survival and consequently wildlife management objectives. Our objectives were to determine the pathogenicity of a common strain of *P. haemolytica* from healthy domestic sheep in healthy bighorn sheep and healthy domestic sheep, and to characterize the isolates of *P. haemolytica* with a genomic fingerprinting technique known as ribotyping.

MATERIALS AND METHODS

Three experiments were done with bighorn sheep (*O. canadensis canadensis*) and domestic sheep between August 1991 and January 1992

at Washington State University (WSU), Pullman, Washington (USA). All animals were clinically healthy at the initiation of each experiment.

Microbiology techniques

A flock of 37 healthy domestic sheep at the University of Idaho, Moscow, Idaho (USA) was sampled for *P. haemolytica* by inserting sterile cotton-tipped swabs into the nares of each sheep. Swabs were placed in Amies transport medium (Spectrum Diagnostics, Inc., Houston, Texas, USA), transported to the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington, and streaked onto 5% sheep blood agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) within 2 hr of collection. Isolation and identification of *P. haemolytica* was accomplished by the methods of Snipes et al. (1992), but hemolysis on 5% sheep blood agar or growth on MacConkey's agar (Becton Dickinson Microbiology Systems) were not requisites for identification (Onderka et al., 1988; Wild and Miller, 1991). All *P. haemolytica* isolates were identified to serotype by rapid plate agglutination (Frank and Wessman, 1978). Two separate isolates of *P. haemolytica* biotype A, serotype 2, from two healthy domestic sheep were chosen initially for this experiment because it is the most common serotype isolated from healthy and pneumonic domestic sheep (Thompson et al., 1977; Frank, 1982). Both isolates (numbers 1 and 2) were frozen in phosphate buffered glycerol at −70 C until they were used. Cultures for inoculation were grown in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Michigan, USA) at 37 C for approximately 16 hr before inoculation. Estimated concentration of bacteria in the BHI broth culture just prior to inoculation was determined by the McFarland Method (Balows et al., 1991), and final concentration of *P. haemolytica* in the inoculum was measured in colony forming units (CFU) per ml by culturing ten-fold serial dilutions retrospectively after inoculations had been given. While animals were restrained physically, the inoculations were injected intratracheally with a 3 cc syringe and 20 gauge needle.

Cultures of each *P. haemolytica* isolate obtained during the experiments were frozen in phosphate buffered glycerol at −70 C until evaluation by a genomic fingerprinting technique known as ribotyping (Snipes et al., 1992). Isolates were regrown on 5% sheep blood agar plates, and the DNA was obtained from each isolate using the method of Wilson (1987). After the bacterial cells were lysed with sodium dodecyl sulfate, proteins and other cellular debris were removed by digestion with proteinase-K and precipitation with hexadecyltrimethyl ammonium bromide, and the DNA was precipitated with isopropanol (Snipes et al., 1992). The concentration of DNA in each sample was determined fluorometrically (DNA fluorometer model TKO 100, Hofer Scientific Products, San Francisco, California, USA) and 3.0 μg of purified DNA from each isolate were digested for 2 hr at 37 C with approximately 10 units of the restriction enzyme *Eco*RI in a 30 μl reaction mixture containing the appropriate buffer (Snipes et al., 1992). Electrophoresis of the sample was done in a 0.7% horizontal slab agarose gel and stained with ethidium bromide (Snipes et al., 1989). Digested DNA fragments were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, New Hampshire, USA) using methods of Southern (1975). The DNA blots were dried at 80 C in a vacuum oven and stored at 20 C.

Escherichia coli rRNA (Sigma Chemical Co., St. Louis, Missouri, USA) was labelled with ^{32}P-phosphate and hybridized with restriction enzyme-digested and blotted *P. haemolytica* DNA using methods described by Snipes et al. (1989). Specific activity of the probe was approximately 10^7 counts per minute (cpm) per μg of RNA, and blots were hybridized with approximately 10^6 cpm/ml of hybridization solution. Extent of hybridization of the probe with blotted DNA was analyzed by autoradiography (Snipes et al., 1989), and profiles of the *P. haemolytica* isolates were compared visually and grouped based on restriction enzyme fragment patterns. Ribotype patterns were also digitized for comparison using a digitizing tablet and the MolMatch computer software (UVP Inc., San Gabriel, California) for DNA and protein-band matching.

At the beginning of each experiment, nasal swab samples (Marion Scientific Viral Culturette, Marion Scientific, Kansas City, Kansas, USA) were collected for virus evaluation. Specimens were inoculated onto ovine fetal tracheal cells and bovine turbinate cells for two passages at 10-day intervals and were examined daily for cytopathic effect (Castro, 1992). Additional specimens were tested for respiratory syncytial virus by use of solid phase-enzyme immunoassay (Abbott RSV EIA, Abbott Laboratories, South Pasadena, California). Isolation of *Chlamydia* spp. was not attempted. Fecal samples from 10 of 13 bighorn sheep were evaluated for lungworm larvae by a modified Baermann technique (Beane and Hobbs, 1983).

Experiment 1

Four bighorn sheep and two domestic sheep were used (Fig. 1). The bighorn sheep, all 2.5-yr-old castrated males, were captive-born in a herd in Fort Collins, Colorado (USA), trans-

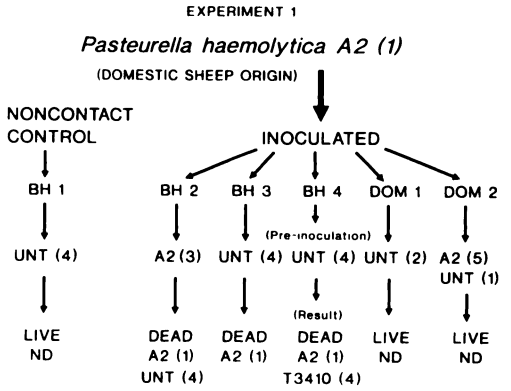

FIGURE 1. Experimental design of experiment 1 indicating isolates of *Pasteurella haemolytica* made before and after inoculation; preinoculation refers to isolates of *Pasteurella haemolytica* isolated from sheep prior to inoculation. Numbers in parentheses indicate WSU *Eco*RI ribotype reference number, numbers indicate serotype, A = biotype A, T = biotype T, UNT = untypeable serotype, ND = not done, BH = bighorn sheep, DOM = domestic sheep. Numbers following BH and DOM are identification numbers of individual animals.

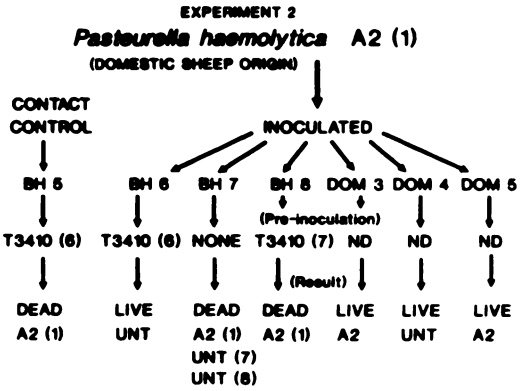

FIGURE 2. Experimental design of experiment 2 indicating isolates of *Pasteurella haemolytica* made before and after inoculation; preinoculation refers to isolates of *Pasteurella haemolytica* isolated from sheep prior to inoculation. Numbers in parentheses indicate WSU *Eco*RI ribotype reference number, numbers indicate serotypes, A = biotype A, T = biotype T, UNT = untypeable serotype, ND = not done, BH = bighorn sheep, DOM = domestic sheep. Numbers following BH and DOM are identification numbers of individual animals.

ported to WSU, and held for approximately 1 yr before initiation of this experiment. The domestic sheep were 9-mo-old wether lambs maintained as normal blood donors with 10 other sheep at WSU. The healthy domestic sheep were maintained on a 1-ha pasture and fed alfalfa hay. Trace mineral salt and water were available at all times. All four bighorn sheep were maintained on a 2-ha pasture, and contact with other ruminants was not allowed. Natural grasses were abundant, and supplemental alfalfa pellets, trace mineral salt, and water were available at all times.

On the day of inoculation, pharyngeal swab samples were collected from all bighorn sheep. A speculum was used to hold the mouth open and restrain the tongue. A sterile polyester tipped applicator swab was used to rub the pharyngeal area briskly, removed, and placed in transport medium (Port-A-Cul® tube, Becton Dickinson Microbiology Systems) for transport to WADDL for evaluation. All pharyngeal swabs were streaked on sheep blood agar plates within 2 hr of collection to maximize isolation of *P. haemolytica* (Wild and Miller, 1991).

After collection of pharyngeal samples, bighorn sheep number 1 was injected intratracheally with 2 ml of sterile BHI broth. Bighorn sheep number 2 and domestic sheep number 1 each were inoculated with 6.0×10^{11} CFU of *P. haemolytica* A2 of domestic sheep isolate number 1, and bighorn sheep numbers 3 and 4, and domestic sheep number 2 were each inoculated with 8.6×10^{11} CFU of *P. haemolytica* A2 of domestic sheep isolate number 2.

Experiment 2

Four bighorn sheep, two 2-yr-old ewes and two 2-yr-old rams, and three domestic sheep were used (Fig. 2). All four bighorn sheep had been in captivity for ≥1 yr before the experiment, and were maintained together on a 1-ha pasture. Trace mineral salt, alfalfa hay, alfalfa pellets and water were available at all times. The domestic sheep were 1-yr-old wethers and were held on the same pasture and with the same flock of domestic sheep as described in experiment 1. On the day of inoculation, pharyngeal swab specimens were collected from all bighorn sheep as described in experiment 1. Bighorn sheep number 5 was injected intratracheally with 2 ml of sterile BHI broth; bighorn sheep numbers 6, 7, and 8 were inoculated with 0.25 ml (0.75×10^{11} CFU), 0.75 ml (2.25×10^{11} CFU) and 2.0 ml (6×10^{11} CFU) respectively, of a bacterial suspension of domestic sheep isolate number 1 from the same original culture used in experiment 1. Domestic sheep numbers 3, 4, and 5 each were inoculated intratracheally with 2 ml (6×10^{11} CFU) of the same bacterial suspension.

Experiment 3

Five bighorn sheep, including three 8-mo-old lambs (two females, one male), a 2-yr-old ewe,

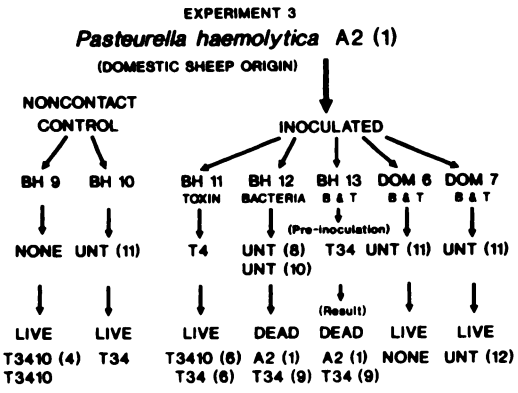

FIGURE 3. Experimental design of experiment 3 indicating isolates of *Pasteurella haemolytica* made before and after inoculation; preinoculation refers to isolates of *Pasteurella haemolytica* isolated from sheep prior to inoculation. Numbers in parentheses indicate WSU *Eco*RI ribotype reference number, numbers indicate serotypes, A = biotype A, T = biotype T, UNT = untypeable, B&T = bacteria and toxin, BH = bighorn sheep, DOM = domestic sheep. Numbers following BH and DOM are identification numbers of individual animals.

and a 3-yr-old castrated male, and two 10-mo-old female domestic sheep were used (Fig. 3). The bighorn sheep were maintained on a 1 ha pasture as described in experiments 1 and 2. The three domestic sheep were maintained in the flock described in experiments 1 and 2. On the day of inoculation, pharyngeal swab samples were collected from all sheep and evaluated as described in the previous experiments.

Two bighorn sheep (numbers 9 and 10) each received 2 ml of sterile BHI broth only. Bighorn sheep number 11 received only 2 ml of BHI broth with bacterial supernatant containing toxin, but no bacteria. The toxin was separated from the bacteria after 16 hr of growth by centrifuging the inoculum at 6,000 × *G* and filtering the supernatant through a 0.2 μm filter. Bighorn sheep number 12 was inoculated intratracheally with 5.3×10^{8} CFU of domestic sheep isolate number 1 that had been centrifuged and separated from the supernatant, and resuspended with 2 ml of sterile BHI broth just before inoculation. Bighorn sheep number 13 was inoculated with 2 ml (5.3×10^{8} CFU) of the bacterial suspension of domestic sheep isolate number 1. Domestic sheep numbers 6 and 7 each were inoculated with 2 ml (5.3×10^{8} CFU) of the same bacterial suspension.

Evaluation

All sheep were observed three times daily for signs of respiratory disease for the first week after inoculation, and once daily for 30 days thereafter. If sheep developed respiratory disease, they were humanely euthanized with an intravenous injection of 30 g of pentobarbital sodium (Anthony Products Company, Arcadia, California). All dead sheep were submitted to WADDL for complete necropsy evaluation. Bacterial isolations on blood agar were attempted from the tonsil, bronchial lymph nodes, spleen, and lungs. Representative tissues were fixed in 10% buffered formalin, sectioned at 5 μm, and stained in hematoxylin and eosin for microscopic evaluation. A pharyngeal swab sample was collected from most surviving bighorn sheep and from three domestic sheep between 3 and 7 days after inoculation (Figs. 1, 2, 3).

RESULTS

Both inoculation strains of *P. haemolytica* from domestic sheep were identified as biotype A, serotype 2, ribotype reference WSU-1, hereafter referred to as A2 (1). The inoculation strain was not recovered from any bighorn sheep before inoculation.

Seven of eight bighorn sheep inoculated with 5.3×10^{8} to 8.6×10^{11} CFU of *P. haemolytica* A2 (1) died from acute pneumonia within 48 hr of inoculation (Figs. 1, 2, 3). Bighorn sheep number 6, inoculated intratracheally with 7.5×10^{10} CFU, survived. All bighorn sheep that died were found dead during the first observation period in the morning. Two of the bighorn sheep that died had increased respiratory rates the evening before death, but were not debilitated. The other five bighorn sheep that died appeared healthy on the evening before death. One bighorn sheep (BH 5) that was inoculated only with sterile BHI also died from pneumonia 6 days after its penmates were inoculated with bacteria (Fig. 2). In all dead bighorn sheep, the inoculation strain of *P. haemolytica* A2 (1) was recovered from lung, bronchial lymph nodes, spleen or tonsil. All domestic sheep, including inoculated and contact animals, remained clinically healthy during the experiment.

Twelve distinct ribotypes (groups) of *P. haemolytica* were identified from bighorn

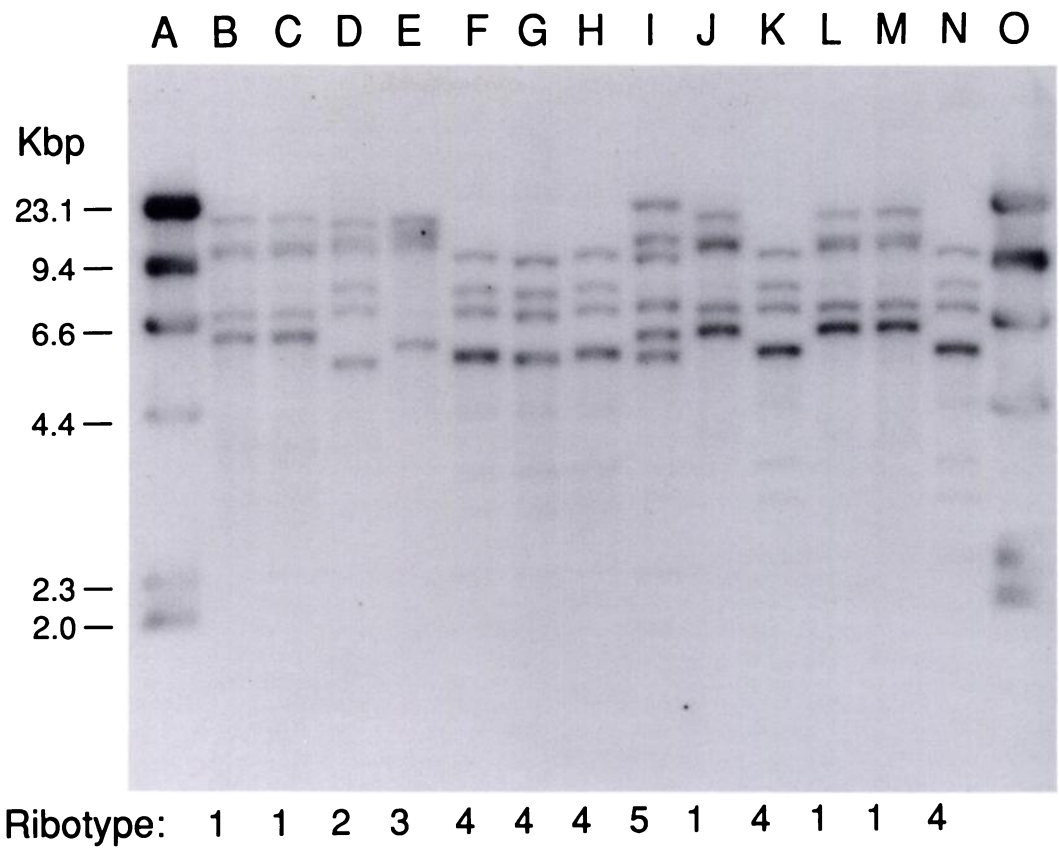

FIGURE 4. Autoradiograph of Southern blot of *Eco*RI-digested DNA from 13 *Pasteurella haemolytica* isolates after hybridization with a ^{32}P labelled *E. coli* rRNA probe. Patterns in lanes B to M represent different ribotype groups as follows: Lanes B, C, D, I, and J represent strains of *P. haemolytica* from domestic sheep; lanes E, F, G, H, K, L, M, and N represent strains from bighorn sheep. Lanes A and O represent DNA isolated from lamda phage, digested with *Hind*III and hybridized with *Hae*III-digested lambda phage probe. Numbers on the left represent the number of kilobase pairs (kbp) of the representative lambda phage fragments.

and domestic sheep during these experiments. Representative ribotypes are illustrated in Fig. 4, and digitized representatives of all 12 ribotypes are shown in Fig. 5. Ribotypes were determined for 43 isolates from the three experiments, and eight additional isolates did not grow on subsequent culture or were not evaluated for ribotype. Of the 43 isolates that were evaluated for serotype, 18 (42%) were *P. haemolytica* A2 (1) which was recovered only from the inoculum and from dead bighorn sheep (Figs. 1, 2, 3). An additional isolate of *P. haemolytica* from domestic sheep number 2 before inoculation was untypeable for serotype, but was WSU ribotype reference 1. For this experiment, ribotype reference WSU-1 was characterized with the following kilobase pair fragments: 26.82 (light), 15.01, 11.15, 10.61, 7.87 (light), 6.97, and 6. Two additional isolates of *P. haemolytica* A2 included ribotypes 3 and 5 (Fig. 1).

Of the 43 isolates evaluated for ribotype, 14 (33%) were untypeable for serotype. These 14 untypeable isolates represented eight different ribotypes (WSU ribotypes 1, 2, 4, 7, 8, 10, 11, 12), including an isolate of ribotype reference WSU-1 from domestic sheep number 2 before inoculation.

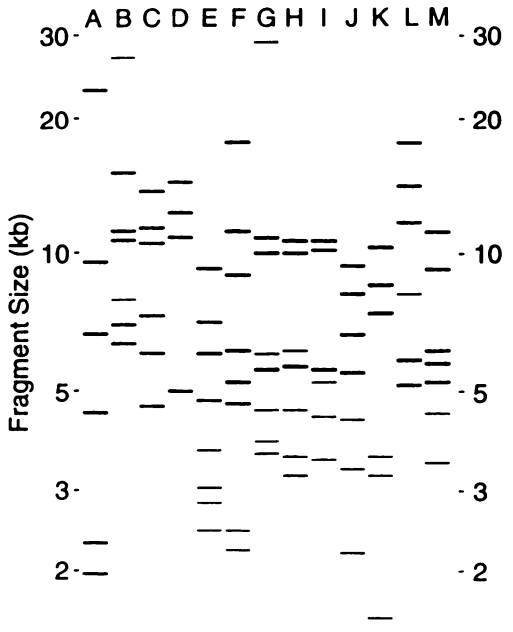

FIGURE 5. Digitized representations of all 12 *Pasteurella haemolytica* WSU *Eco*RI ribotype patterns observed in the study. Lane A = lambda phage, digested with *Hind*III and hybridized with *Hae*III-digested lamda phage probe; Lanes B through M represent ribotypes 1 through 12, respectively.

Six of the isolates cross-reacted with serotypes T3, 4, and 10, and three isolates cross-reacted with serotypes T3 and 4. Four different ribotypes (ribotypes 4, 6, 7, 9) were represented in this group of cross-reacting serotypes (Figs. 1, 2, 3). No viruses were isolated from any of the sheep. Less than ten *Protostrongylus* sp. larvae per gram of feces were recovered from 4 of 10 bighorn sheep; lungworm larvae were not detected from the other six bighorn sheep.

At necropsy, all bighorn sheep were in good body condition with adequate amounts of body fat. Lesions were similar in all bighorn sheep and characteristic of acute, fibrinohemorrhagic pneumonia and pleuritis. Up to 70% of lung volume was dark red and consolidated with small to moderate amounts of adherent fibrin. On cut surface, lungs were diffusely edematous with prominent interlobular septa. Regional lymph nodes (mandibular, cervical, tracheobronchial, mediastinal) were enlarged.

Histologically, pulmonary architecture was diffusely and severely altered by large areas of necrosis marginated by densely packed or clumped neutrophils and macrophages. The pleura was markedly thickened by fibrin deposits, and subpleural spaces plus interlobular septa were widened by collections of fluid and exudate. Densely basophilic bacterial colonies were mixed with the cellular exudates, especially in terminal bronchioles and remaining air spaces. Adjacent alveolar capillary endothelium was disrupted, and fibrin thrombi were common within these blood vessels.

DISCUSSION

There was clear evidence of an increased susceptibility of bighorn sheep to *P. haemolytica* A2 (1) when compared to domestic sheep. Seven of eight inoculated bighorn sheep died from acute pneumonia within 48 hr after inoculation, whereas the domestic sheep that received comparable or greater amounts of bacteria at the same time remained healthy. Silflow et al. (1991) compared arachidonic acid metabolism in alveolar macrophages of domestic sheep and bighorn sheep and found that bighorn sheep had lower levels of 5(S)-hydroxyeicosatetraenoic acid (5-HETE) and 5(S), 12(R)-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid (LTB4), indicating a possible mechanism for increased susceptibility to respiratory disease.

At least 15 known serotypes of *P. haemolytica* have been documented (Tsai et al., 1988) as well as several other serotypes that are not typeable or cross react with two or more serotypes (Snipes et al., 1992). *Pasteurella haemolytica* A2 was selected for these experiments because it is the most common biotype and serotype of *P. haemolytica* carried by domestic sheep (Frank, 1982), and is one serotype that likely would be transferred to bighorn sheep when close contact occurs between bighorn and domestic sheep. Although both domestic

sheep isolates of *P. haemolytica* A2 (1) used in these experiments resulted in fatal pneumonia in bighorn sheep, effects of the other serotypes and ribotypes of *P. haemolytica* in bighorn sheep are unknown.

Determining ribotypes of *P. haemolytica* as a supplement to biotyping and serotyping may have several benefits in evaluating results of experimental bacterial transfer experiments, as well as for use in tracking bacterial transfer between animal species under field and captive situations (Jaworski et al., 1993). In our experiments, three distinct ribotypes of *P. haemolytica* A2 (ribotypes 1, 3, and 5) were isolated from two domestic sheep and eight bighorn sheep (Figs. 1, 2, 3); however, only the inoculum strain of A2 (1) was isolated from dead bighorn sheep, lending strong evidence that the inocula caused the fatal pneumonias that were observed. In addition, the contact control bighorn sheep (BH 5) in experiment 2 which had been inoculated only with sterile BHI, died from pneumonia 6 days after its penmates received the inoculum, and *P. haemolytica* A2 (1) was isolated at necropsy from several tissues, indicating likely transmission of the inoculum strain from inoculated bighorn sheep to the contact control.

The ability to detect several ribotypes within a serotype (Figs. 1, 2, 3), further illustrates the value of ribotyping for separating strains of *P. haemolytica* within the same serotype or from untypeable serotypes. In experiment 1, an untypeable *P. haemolytica* was isolated from domestic sheep number 2 before inoculation. The ribotype was reference 1, further illustrating the benefit of ribotyping. Evidence of several strains occurring within serotypes have been reported for *Pasteurella multocida* (Snipes et al., 1989, 1990) and *P. haemolytica* (Snipes et al., 1992). Based on these data and data from other studies (Snipes et al., 1992; Jaworski et al., 1993), DNA fingerprinting methods must be used to separate strains of *P. haemolytica* within a serotype to allow for meaningful interpretation of data.

Numerous ribotypes of *P. haemolytica* have been isolated and identified from bighorn sheep (Snipes et al., 1992), but the *P. haemolytica* A2 (1) used in our experiment has not been identified previously (K. M. Snipes, unpubl.). To date, however, ribotypes have been compared only within individual experiments. Comparisons among experiments are limited by lack of tools for quantitative comparisons of isolates. Consequently, it is not currently known if *P. haemolytica* A2 (1) is found in healthy bighorn sheep, or whether different virulence factors are present within the same ribotype.

Onderka et al. (1988) inoculated two bighorn sheep intratracheally with a mixture of *P. haemolytica* from three domestic sheep at a concentration of 2×10^{12} organism. Only one isolate in the composite inoculum, a T15, was serotyped. Both inoculated bighorn sheep died from bronchopneumonia within 48 hr after inoculation in that study. At necropsy, T4 was isolated from one sheep, and T15 was isolated from the other. In the current experiment, only one DNA ribotype was used in the inoculum to focus on a specific ribotype found in domestic sheep. Based on our data, *P. haemolytica* A2 (1) appears lethal in bighorn sheep at the concentrations used in this study.

Immunity to *P. haemolytica* in cattle is not completely understood (Confer et al., 1988), and in bighorn sheep is essentially unknown. The origin of the bighorn sheep used in the first experiment was a captive herd in Colorado that had a history of chronic pasteurellosis (Miller et al., 1991). Although the ribotypes present in that herd were different from the inoculum used in this experiment (Snipes et al., 1992; K. P. Snipes, unpubl.), all inoculated bighorn sheep died; thus they probably lacked resistance. Few data are available on whether nonpathogenic strains of *P. haemolytica* found in healthy bighorn sheep induce protection against pathogenic strains. Based on our experiment, strains of *P. haemolytica* carried by the bighorn sheep did not

prevent death when the bighorn sheep were inoculated intratracheally with the domestic sheep strain of *P. haemolytica* A2 (1).

The role of domestic sheep in the epizootiology of bighorn sheep pneumonia is an important issue in wildlife management and multiple land use. Elimination of domestic sheep contact with bighorn sheep would theoretically eliminate the direct transmission of pathogens between species and the mortality which may be attributable to specific domestic sheep strains of *P. haemolytica*. *Pasteurella haemolytica* is a relatively labile bacterium and generally requires direct physical contact between animals for transmission. Although the numbers of bacteria used in these experiments may not accurately reflect a natural exposure, nevertheless, one contact control bighorn sheep died within six days after its penmates received the inocula, and healthy domestic sheep inoculated with equal or greater numbers of bacteria remained healthy after inoculation indicating greater susceptibility of bighorn sheep to infection and fatal pneumonia. Because *P. haemolytica* A2 is a common isolate in domestic sheep, the likelihood of transmission from domestic sheep to bighorn sheep would be significant in contact situations. Based on these and previously reported data, all contact between bighorn sheep and domestic sheep should be avoided to prevent transmission of pathogenic strains of *P. haemolytica* of domestic sheep origin that cause pneumonia in bighorn sheep. Whether similar pathogenic strains of *P. haemolytica* exist in healthy bighorn sheep populations is unknown. Future data that compare biotype, serotype, ribotype and cytotoxicity of *P. haemolytica* isolates (Silflow et al., 1993), and experimental animal inoculation data, may help wildlife managers predict mortality and long term population dynamics of bighorn sheep populations, especially in areas where contact with domestic sheep is likely, or where bighorn sheep populations historically undergo cyclical losses due to pneumonia.

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