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FAILURE OF AN EXPERIMENTAL *PASTEURELLA HAEMOLYTICA* VACCINE TO PREVENT RESPIRATORY DISEASE AND DEATH IN BIGHORN SHEEP AFTER EXPOSURE TO DOMESTIC SHEEP

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Abstract: An experimental bacterin-toxoid vaccine of *Pasteurella haemolytica* A2, T3, and T10 was evaluated in bighorn sheep (*Ovis canadensis canadensis*). Three bighorn sheep were vaccinated twice, 14 days apart, and 3 bighorn sheep were not vaccinated. Challenge infection was accomplished by introducing 4 clinically healthy domestic sheep that had detectable *P. haemolytica* in nasal sinuses onto the 2.4 ha pasture with the bighorns. Five of 6 bighorn sheep, including the 3 vaccinates died within 40 days after exposure to domestic sheep. *Pasteurella* sp., *P. haemolytica* T3,4,10,11, an untypeable *P. haemolytica*, and *P. multocida* were isolated from dead bighorn sheep, and *P. haemolytica* A1, A2, and *P. multocida* were isolated from the domestic sheep. Vaccine efficacy could not be determined, as *Pasteurella* spp. in dead bighorns differed from those in the vaccine. However, the vaccine did not protect the vaccinated sheep from clinical pneumonia and death. The experiment reinforced the premise that domestic sheep and bighorn sheep should be separated or bighorns may die from pneumonia.

Respiratory disease is a major mortality factor in bighorn sheep populations in North America (Buechner 1960, Spraker and Hibler 1982). Predisposing factors, such as lungworms (*Protostrongylus* spp.), respiratory viruses and bacteria, and various stressors, are often associated with bighorn sheep pneumonia (Post 1962, Forrester 1971, Spraker and Hibler 1982, Onderka and Wishart 1984, Spraker et al. 1984), however, the major organism isolated from pneumonic bighorns is *P. haemolytica*, a gram negative pneumophylic bacterium (Coggins 1988, Onderka and Wishart 1984, 1988, Foreyt 1989). Two major biovars, A and T, serovars 1 through 15, and several untypeable serovars are part of the *P. haemolytica* complex. The T biotype, which is often nonhemolytic on blood agar, is isolated commonly from pneumonic and clinically healthy bighorn sheep (Onderka et al. 1988, Wild and Miller 1991).

Contact with domestic sheep is an important predisposing factor for some pneumonia episodes in bighorn sheep. Under experimental and field conditions, high mortality rates have occurred for bighorn sheep after such contacts (Coggins 1988, Onderka and Wishart 1988, Onderka et al. 1988, Foreyt 1989, 1990). Serotypes of *P. haemolytica* that are usually nonpathogenic in domestic sheep are likely transferred to bighorn sheep, resulting in fatal pneumonia in bighorn populations (Foreyt and Jessup 1982, Onderka and Wishert 1988, Foreyt 1989, 1990). It is also probable that recruitment in residual bighorn sheep populations surviving *P. haemolytica* pneumonia after domestic sheep association or from indigenously acquired pneumonia will be low for several years (Bailey

1986, Coggins 1988, Foreyt 1990). Lambs likely acquire P. haemolytica from oral and nasal secretions from their dams, and die from pneumonia at 6-11 weeks of age when colostral immunity wanes (Foreyt 1990). Although the exact mechanism which is responsible for pneumonia in bighorn sheep following association with domestic sheep is not known, experimental and field data indicate that bighorn sheep and domestic sheep are not compatible species on the same range. Physiologically, bighorn sheep alveolar macrophage function and arachidonic acid metabolism differ significantly from domestic sheep and may be factors in the increased sensitivity of bighorn sheep to respiratory disease compared to domestic sheep (Silflow et al. 1991).

This study evaluated the efficacy of an experimental P. haemolytica bacterin-toxoid vaccine against respiratory disease in bighorn sheep by placing domestic sheep on the same pasture with vaccinated and unvaccinated bighorn sheep. Clinical pneumonia and mortality were the major parameters used to determine effectiveness of the vaccine.

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MATERIALS AND METHODS

Six Rocky Mountain bighorn sheep (Ovis canadensis canadensis), 3 males and 3 females, ranging in age from 1 to 5 years, were used. Five of the sheep were born and raised in captivity at Washington State University, Pullman, Washington, and 1 sheep (No. 5) was captured in the wild and held for 6 months with the other 5 sheep before the experiment began. Fresh water and a shelter were available at all times, but supplemental feed was not provided because pasture forage conditions on the 2.4 ha pasture were excellent.

Nasal swabs were collected from each bighorn 5 months prior to initiation of the experiment, and at the time of the first vaccination. Sheep were sampled for bacteria by inserting cotton tipped swabs into the nares of each sheep, and immediately placing them in Stuart's transport medium without agar. Swabs were submitted within 2 hours after collection to the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington 99164, for bacteriologic analysis. Bacterial isolates were confirmed as P. haemolytica by routine biochemical testing (Carter 1984). Biotyping and rapid plate serotyping methods were done according to established formats (Biberstein 1978, Frank and Wessman 1978).

Three bighorn sheep, a 3 yr-old and a 5 yr-old female, and a 1 yr-old male, were not vaccinated, and 3 bighorn sheep, 2 3-yr-old males, and a 3 yr-old female were vaccinated intramuscularly with 2 mls of an experimental P. haemolytica vaccine (NOBL Laboratories, Sioux Center, Iowa 51250) on 10 and 24 April 1990. The formalinized vaccine was a bacterin-toxoid prepared from leucotoxoid and outer membrane proteins of P. haemolytica, types A2, T3, and T10. The original strains of P.

haemolytica were isolated from bighorn sheep that died from pneumonia after exposure to domestic sheep (Foreyt 1990). Types A2, T3, and T10 antigen components were prepared in identical fashion until batching into the vaccine. Each component was grown in RPMI-1640 containing L-glutamine. Bovine serum albumin was supplemented at 0.1%. Final bulk harvest was 6 passages beyond the original isolate. Growth was at 37 C in a glass bottle, and cultures were stirred at a moderate speed. Cultures were harvested during log phase at 6 hours and found to be well encapsulated. Fluids were centrifuged and the cells separated from the supernatent. Fluids were concentrated approximately 10 times using an Amicon unit equipped with a Y-10 filter. Formalin was added to a concentration of 0.5% (v/v) and the mixture stirred at 4 C for 48 hr. This leukotoxoid was adsorbed to 10% aluminum hydroxide (v/v). The cell fraction was resuspended in normal saline and sonicated until capsules were no longer evident by microscopic examination. The sonicate was centrifuged and the supernate retained. The leukotoxoid and the crude outer membrane proteins were combined with an oil adjuvant for administration.

On 10 April 1990, 28 domestic sheep at the University of Idaho Sheep Center, Moscow, Idaho, were sampled for bacteria using nasal swabs as described previously for bighorn sheep. Duplicate swabs were collected and submitted for viral isolation. Routine laboratory isolation techniques were used for isolation of aerobic bacteria and viruses. Isolation of Chlamydia spp. and Mycoplasma spp. was not specifically attempted. Four domestic sheep, identified as carriers of P. haemolytica, were purchased and introduced into the bighorn sheep pen on 8 May 1990, 2 weeks after the second bighorn vaccination. A second nasal swab was collected 30 days after the first.

Fecal samples were collected from the rectum of all animals at the initiation of the experiment, and from dead animals that were necropsied. A Baermann apparatus was used to isolate larvae from feces, and the sediment was examined microscopically for lungworm larvae. All sheep were observed twice daily for clinical signs of disease. Sheep were to be euthanized if they became clinically affected. A complete necropsy, with emphasis on isolation of respiratory pathogens, was done on each dead sheep. Standard necropsy, histolopathogic, parasitologic, and microbiologic techniques were used by WADDL personnel.

RESULTS

Pasteurella haemolytica was not isolated from nasal swabs from any of the bighorn sheep before or at the time of vaccination. From the 4 domestic sheep, P. haemolytica A1 was isolated from 2, P. haemolytica A2 from 3, and P. multocida from 3 (Table 1). Some coliform bacteria, Streptococcus sp., Bacillus sp. and Pseudomonas sp., were isolated from 1 or more of the bighorn sheep, but were not considered important.

Five of the 6 bighorn sheep, including the 3 vaccinates, died between 26 and 40 ($\bar{x}$ = 34) days after exposure to the domestic sheep. All 5 bighorn sheep that died developed a clinical syndrome of tachypnea, dyspnea, incoordination, and weakness prior to death. Sheep developed clinical signs within 24 hr of death. All bighorn sheep died during the

Table 1. Summary of *Pasteurella* spp. isolated from domestic sheep and bighorn sheep during a vaccine trial.

Sheep number	Age	Sex	Day of death	Nasal swab isolations: Experimental day 0	Nasal swab isolations: Experimental day 30	Lung isolations at death
Vaccinated bighorns						
1	3	F	26	None Isolated	NA[a]	*Pasteurella* sp.[b]
2	3	M	33	None Isolated	ND[c]	*P. haemolytica*[d] (untypeable) *P. multocida*
3	3	M	34	None Isolated	ND	*P. multocida*
Unvaccinated bighorns						
4	5	F	35	None Isolated	ND	*P. multocida*
5	1	M	40	None Isolated	ND	*P. haemolytica* T3,4,10,11
6	3	F	NA	None Isolated	ND	NA
Domestic sheep						
7	Adult	F	NA	*P. haemolytica* (untypeable)	*P. haemolytica* A2 *P. multocida*	NA
8	Adult	F	NA	*P. haemolytica* A1 *P. multocida*	*P. haemolytica* A1 *P. haemolytica* (untypeable)	NA
9	Adult	F	NA	*P. haemolytica* A1	*P. multocida* A2	NA
10	Adult	F	NA	*P. haemolytica* A2	*P. haemolytica* A2	NA

[a]NA = Not applicable.
[b]Also *Pasteurella* sp. from tracheobranchial lymph nodes.
[c]ND = Not done.
[d]Also *P. multocida* and *P. haemolytica* T3,4,10,11 from tracheobronchial lymph nodes.

night and were found in the morning. At necropsy, all bighorns had adequate body fat and had lesions which were typical of severe hemorrhagic, necrotizing bacterial pneumonia. Grossly, lung lobes were dark red, firm, friable, and consolidated, and were often covered with fibrin tags. Up to 90% of lung parenchyma was involved with fibrous adhesions between lung lobes, pericardium, and parietal pleura. Histopathologic pulmonary lesions were consistent with bacterial pneumonia and included marked accumulation of neutrophils, macrophages, cellular debris, and proteinaceous fluid. The pleura was thickened and disrupted by infiltrates of histiocytes, lymphocytes, and neutrophils. Fibrin and necrotic debris replaced much of the pulmonary tissue.

Bacterial isolates from the vaccinated bighorns included Pasteurella sp. (species could not be identified) from lung and tracheobronchial lymph node of bighorn No. 1, an untypeable P. haemolytica and P. multocida from lung, and P. multocida and P. haemolytica (cross reacted with T3,4,10, and 11) from tracheobronchial lymph node of bighorn No. 2, and P. multocida from lung of bighorn No. 3 (Table 1). The 2 unvaccinated bighorn sheep that died yielded P. multocida and P. haemolytica (cross reacted with T3, T4, T10, and T11) from lung of No. 4, and P. multocida from lung of No. 5 (Table 1). Viruses were not isolated, lungworms were not detected in lungs, and lungworm larvae were not detected in feces or histologically in any bighorn sheep. The last bighorn survived the experiment and remained healthy for 12 months after the termination of the experiment.

All 4 domestic sheep remained clinically healthy during the 60 days they were on the pasture with the bighorn sheep. Viruses, lungworms, or lungworm larvae were not isolated from them.

DISCUSSION

Five of 6 bighorn sheep developed clinical pneumonia and died 26-40 days after exposure to domestic sheep, supporting previous reports that association with domestic sheep may predispose bighorn sheep to fatal pneumonia (Foreyt and Jessup 1982, Coggins 1988, Onderka and Wishart 1988, Foreyt 1989, 1990). In previous reports, P. haemolytica has been incriminated as the major pathogen transmitted from domestic sheep. In this experiment, P. multocida was the only pathogen isolated from lungs of 2 of the bighorn sheep (1 vaccinate and 1 nonvaccinate), and was also isolated with P. haemolytica in 2 others, supporting the results of Callan et al.(1991), who isolated P. multocida from 5 of 6 dead pneumonic bighorns after exposure to a flock of exotic wild and domestic sheep. Based on all published reports, it is likely that some strains of P. haemolytica and P. multocida are pathogenic to bighorns after transfer from domestics.

Vaccinated and unvaccinated sheep died within the same time period, 26 to 40 days after exposure to domestic sheep, and lesions in all dead sheep were similar. This indicated that the vaccine did not exacerbate clinical disease. Wilke et al. (1980) reported that calves vaccinated with a P. haemolytica bacterin and then challenged with P. haemolytica, were more severely affected by clinical disease and lesions than were unvaccinated calves.

The only P. haemolytica isolated from vaccinated bighorn sheep lungs was untypeable. A second vaccinated bighorn sheep had a Pasteurella sp. that could not be identified to species. These results involving P. multocida, untypeable P. haemolytica, and uncharacterized Pasteurella sp. further complicate knowledge regarding the epizootiology of the bighorn sheep pneumonia complex and the association with domestic sheep. It is likely that a variety of Pasteurella spp. organisms may be lethal to bighorn sheep, and more reliable diagnostics are needed for bacterial isolation and identification. Bacteria may have similar morphologic characteristics in culture, yet their identities may differ, suggesting that many colonies per agar plate must be identified. Several serotypes of P. haemolytica have been isolated from single morphologic colony types (Onderka et al. 1988), and several DNA types and ribotypes may be present within serovars (Snipes et al. 1992). DNA analysis, ribotype analysis, and cytotoxicity studies may clarify the identification and pathogenicity of organisms important in the bighorn sheep pneumonia complex.

Culture of tonsillar biopsies and pharyngeal swabs from bighorn and domestic sheep has indicated that many bighorn and domestic sheep are carriers of P. haemolytica, even though bacteria cannot be isolated from nasal swabs (Shreeve and Thompson 1970, Gilmour et al. 1974, Al-Sultan and Aitken 1985, Onderka and Wishart 1988, Dunbar et al. 1990, Wild and Miller 1991). Only nasal swabs were used in this experiment; therefore, it is possible the bighorns were carriers of P. haemolytica and/or P. multocida sequestered in pharyngeal tissue, without shedding bacteria in nasal secretions. However, no Pasteurella spp. were isolated from bighorn sheep nasal swabs 5 months before the experiment and on the day of vaccination, indicating that if Pasteurella spp. were present, shedding rate was low.

Possible important factors in the epizootiology of the pneumonia complex include stress from the presence of domestic sheep in close proximity to bighorns, and bacteria from domestic sheep suppressing bighorn immune function, allowing indigenous bacteria to colonize and initiate pathogenic responses. However, based on available data, bacteria transferred from domestic sheep to bighorn sheep likely resulted in bighorn deaths. Unusual human activity, noise, inclement weather, nutritional deficiencies, adverse social encounters, population density factors, or other stressors could not be identified specifically before or during the experiment, but stress in various forms could have occurred. Stress parameters were not eliminated. The bighorn sheep usually segregated from the domestic sheep on the pasture, but occasionally shared common resting and feeding areas, and interacted socially with the domestic sheep. Effects of inapparent stressors that could be important in the epizootiology of bacterial pneumonias in bighorn sheep remain to be evaluated. Although adequate nutrition, minimal population density, and other management factors reduce the probability of disease related die-offs, association with domestic sheep appears to function independently in predisposing fatal pneumonia.

Only 1 bighorn sheep survived the experiment, and remained clinically normal 1 year later. This sheep was born in captivity, and to my knowledge did not experience respiratory disease before, during, or after the experiment. Inherent genetic resistance or acquired immunity are

possible explanations for survival. Acquired immunity or inherent genetic resistance could protect sheep from pneumonia, and could explain bighorn ewes surviving a die-off related to domestic sheep exposure and producing lambs that for several successive years succumb to fatal pneumonia (Coggins 1988, Foreyt 1990).

The vaccine used in this experiment did not protect bighorn sheep against clinical pneumonia and death. However, Pasteurella spp. strains isolated from dead bighorns may not have been in the vaccine, specifically, P. multocida, untypeable P. haemolytica and Pasteurella sp. Therefore, vaccine efficacy could not be evaluated from this experiment, but the vaccine was a failure in terms of preventing sickness and death. Newer vaccines are likely to include many serovars important in the Pasteurella spp. complex. Vaccines incorporating serovars types that have cross protective characteristics would be most effective. An effective vaccine against P. haemolytica and P. multocida in free-ranging bighorn sheep, and possibly domestic sheep, would represent a significant and needed advance in wildlife management by protecting herds from massive die-offs caused by Pasteurella spp., and the deleterious effects of low recruitment following the initial mortality (Bailey 1986, Coggins 1988, Foreyt 1990).

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