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PNEUMONIA IN BIGHORN SHEEP: EFFECTS OF *Pasteurella haemolytica* FROM DOMESTIC SHEEP AND EFFECTS ON SURVIVAL AND LONG-TERM REPRODUCTION

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Abstract: Based on experiments and field observations, pneumonia caused by *Pasteurella haemolytica*, primarily serotypes A_2, T_3, T_4, T_{10}, can be devastating in bighorn sheep. Under controlled conditions, 2 Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) exposed to 2 domestic sheep developed pneumonia and died. Serotypes of *P. haemolytica* isolated from dead bighorns included A_2, $T_{3,4,10}$, and T_{10}. In a second experiment, *P. haemolytica* from domestic sheep was accidentally introduced into a captive herd of *O. c. canadensis* at Washington State University, and resulted in respiratory disease in the 10 sheep, and death in 3 sheep. *P. haemolytica* was isolated from most of these sheep after exposure; whereas, none had detectable *P. haemolytica* from nasal swabs before the accidental induction. Although lambs were born to clinically normal ewes that were shedding *P. haemolytica* in nasal secretions, all lambs died each year for 2 consecutive years after the pneumonia episode. Lambs were healthy until 6-11 weeks of age when they developed pneumonia and died. Lambs were apparently protected for several weeks after birth by passive immunity from colostrum. When this immunity waned, they were overwhelmed by *P. haemolytica* from nasal secretions of their ewes. Two adult bighorn ewes which had no detectable *P. haemolytica* from nasal swabs were added to the herd, and died from pneumonia within a year. A noninfected control herd of *O. c. canadensis* maintained in a different pen at the University had no detectable *P. haemolytica* from nasal swabs, experienced no mortality, and had 100% lamb survival.

Based on these studies, it is likely that recruitment into bighorn sheep herds that survive mortality due to pneumonia caused by *P. haemolytica* will be very low for several years after the initial pneumonia episode. Healthy bighorn sheep introduced into herds that are shedding pathogenic serotypes of *P. haemolytica* are also likely to die. Management recommendations include: 1) exclusion of domestic sheep from contact with bighorn sheep, 2) prohibition of moving healthy bighorns into populations of bighorns that have survived a pneumonia episode or are shedding *P. haemolytica* in nasal secretions, and 3) prohibition of moving survivors from pneumonia episodes, or bighorns shedding *P. haemolytica*, into healthy populations.

Pneumonia is the major mortality factor affecting bighorn sheep in North America. The pneumonia complex is a multi-factorial disease (Fig. 1), often involving several infectious agents incuding lungworms (Protostrongylus); viruses, primarily parainfluenza-3 virus, respiratory syncytial virus and infectious bovine rhinotracheitis virus; and bacteria, primarily Pasteurella haemolytica, P. multocida (Spraker and Hibler 1982, Spraker et al. 1984). Stress can also be important in the pneumonia complex, and can result from adverse environmental conditions, competition with other animals, handling and transportation methods, confinement, interaction with humans, and other factors (Spraker et al., 1984). Depending on circumstances, 1 or more of these factors may predispose to, or cause clinical pneumonia. Certain strains of P. haemolytica apparently are the most important causes of pneumonia in bighorn sheep, and much research has focused on the epidemiology and prevention of pneumonia caused by P. haemolytica.

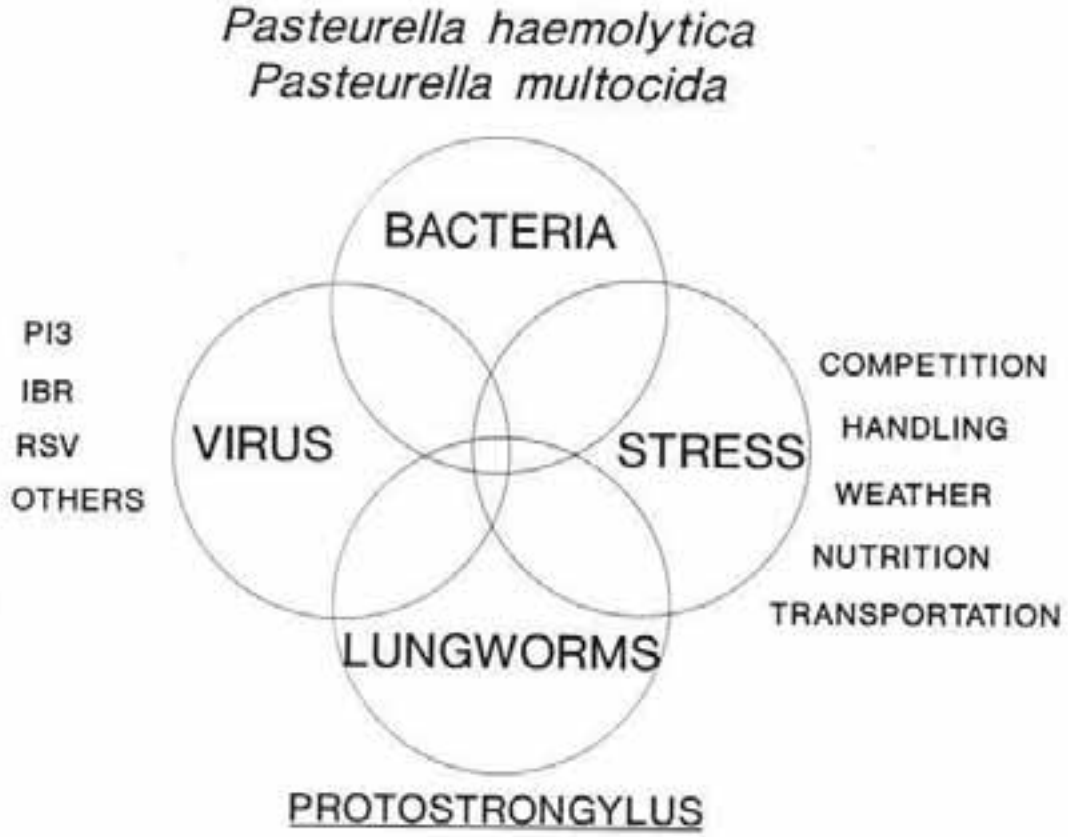

Fig. 1. Causes of the pneumonia complex in bighorn sheep.

Bighorn sheep are highly susceptible to bacterial pneumonia. Epizootics of bacterial pneumonia have decimated several bighorn populations in North America (Onderka and Wishart 1984, Coggins 1988). The domestic sheep-bighorn sheep interaction has become a focus of research because domestic sheep carry strains of P. haemolytica that do not usually affect domestic sheep adversely, but when transferred to bighorn sheep are usually lethal to bighorns (Foreyt and Jessup, 1982; Onderka and Wishart, 1988; Onderka et al., 1988; Foreyt, 1989). The objective of this research was to 1) evaluate the domestic sheep-bighorn sheep association 2) evaluate the short-term and long-term survival of bighorn sheep exposed to bacteria from domestic sheep, and 3) evaluate lamb survival from bighorn sheep ewes that survived a pneumonia episode.

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METHODS

Domestic Sheep – Bighorn Sheep (Experiment 1)

Two bighorn sheep, 1-1/2 year-old rams, were maintained in a 7 x 10m room with concrete walls and floor. Food consisted of alfalfa hay and hay pellets, and mineralized salt and water were provided at all times. The bighorns were born in captivity at Washington State University (WSU) and were well adjusted to captivity and humans. After 3 months of acclimating the bighorns, 2 yearling wether domestic Suffolk sheep, about 6 months old, were introduced into the room. On the day of introduction, nasal swabs were collected from all sheep and evaluated for bacteria by standard laboratory methods. Additional nasal swabs were collected and evaluated on days 13 and 14 after introducing the domestic sheep. When animals died, complete necropsies, including pathology, virology, serology, parasitology, and bacteriology were done by the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington. Standard laboratory methods were used.

Ewe and Lamb Survival (Experiment 2)

In November, 1987, laboratory personnel used a 145cm polyethylene tube, 6mm in diameter, to collect cells from the lungs of several live domestic sheep (Silflow et al. 1989) which had P. haemolytica in nasal secretions as determined from nasal swabs. The serotype was not determined. Following this lavaging procedure, the tube was flushed with saline and the outside of the tube was wiped with 70% alcohol. The same tube was then used within an hour to lavage 3 captive bighorn sheep which were maintained in a 2ha (5 acre) pen containing 7 mature

and 3 immature bighorn ewes. Between 7-12 days following this procedure, all 10 bighorns developed respiratory disease consisting of coughing, increased respiratory rates, and nasal discharges. Sheep were lethargic and anorectic. Three sick sheep were captured and treated with gentamycin and oxytetracycline at 20 mg/kg of body weight, but it was decided that the stress of capture was too detrimental, and treatment was discontinued. In the 3 captured sheep, serum selenium levels were evaluated and considered marginal; therefore, selenium was increased in the mineralized salt from 20 to 90 parts per million. Sheep were observed daily. In December, 1987, an 8-year-old clinically healthy ram from Hall Mountain, Pend Oreille, County, Washington was introduced into the herd, and in January, 1989, 2 clinically healthy adult ewes from northwest Montana were introduced. Two additional bighorns, a ewe and lamb from the same herd in Montana, were introduced into a nonaffected control herd of bighorns (3 rams and 1 ewe) which were maintained on a 2.4ha (6 acre) pasture at WSU. Nasal swabs taken at the times of introduction did not indicate the presence of P. haemolytica in any introduced sheep or in the sheep in the unaffected control pen. Sheep were captured once or twice yearly to collect nasal swabs for bacteria isolations, blood serum for virus antibody analysis, feces for parasite analysis, and for hoof trimming and routine health care.

RESULTS

Domestic Sheep - Bighorn Sheep (Experiment 1)

The domestic sheep remained healthy throughout the experiment. At introduction, P. haemolytica A_1 was isolated from 1, and P. haemolytica T_4 was isolated from the other (Table 1). Both bighorn sheep developed respiratory disease including coughing and rapid respiration on day 13 of the experiment. On day 14, 1 bighorn (OR41) died, and the other (GR 39) was recumbent and moribund, and was euthanatized. Lesions at necropsy were similar in both bighorns and were consistent with an acute, fibrinohemorrhagic, severe, pleuropneumonia of bacterial orgin. Nonhemolytic and hemolytic serotypes of P. haemolytica were isolated from lungs, nasal swabs, thoracic fluid, lavage fluid, lymph nodes and tonsils (Table 1). Serotypes included A_2, $T_{3,4,10}$, and T_{10}. Viruses were not isolated from bighorns or domestic sheep and serology results were unremarkable. Lungworms were not present and other parasite numbers were negligible.

Table 1. Summary of domestic sheep - bighorn sheep experiment.

	DOMESTIC SHEEP	BIGHORN SHEEP
Day 0	Both Normal #1 P. haemolytica A_1 #2 P. haemolytica T_4	Both Normal #1 Negative #2 Negative
Day 13	Both Normal	Respiratory Signs (Both) #1 P. haemolytica (A_2, $T_{3,4,10}$) nasal P. haemolytica (A_2, T_{10}) lavage #2 P. haemolytica (A_2) nasal P. haemolytica (A_2, $T_{3,4,10}$) lavage
Day 14	Both Normal	Both Dead #1 P. haemolytica (A_2, $T_{3,4,10}$) lung P. haemolytica (A_2) thoracic fluid also P. haemolytica tonsil, lymph node #2 P. haemolytica (A_2, $T_{3,4,10}$) lung P. haemolytica ($T_{3,4,10}$) lymph node P. haemolytica (A_2) thoracic fluid also P. haemolytica tonsil

Ewe and Lamb Survival (Experiment 2)

In 1987 following onset of the pneumonia episode, 3 of 10 bighorn ewes, included 2 that had been treated with antibiotics died 6, 18, and 54 days, respectively, after the experimental accident. Lesions were similar and consistent with bronchopneumonia of bacterial origin. Pasteurella haemolytica T_4 was isolated from lung tissue of all 3, and Corynebacterium sp. was isolated from 1. Few lungworm larvae and parasite eggs were detected and other infectious agents

were not isolated. The introduced ram developed respiratory disease approximately 30 days after introduction, but recovered after several months. During fall 1989, the ram became progressively thinner and lethargic, did not shed his summer pelage, and died in November. At necropsy, lesions were consistent with chronic pneumonia, and P. haemolytica was isolated from lungs. Other infectious agents were not isolated. The 2 adult ewes from Montana that were introduced into the herd in January, 1989, died in July and November, respectively, following 2-5 days of respiratory disease. Similar necropsy findings consistent with pneumonia were present, and P. haemolytica was isolated from both ewes, including serotypes A_2, T_4, and $T_{4,10}$. Other infectious agents were not isolated.

Isolates of P. haemolytica from the live adult bighorns are listed in Table 2. Based on analysis of nasal swabs, the P. haemolytica infection declined steadily, but persisted for 2 years in bighorn sheep in Pen 1. In contrast nasal swabs from bighorns in Pen 2 were negative throughout this period (Table 2).

Table 2. Prevalence of Pasteurella haemolytica (number sheep infected/number tested) isolated from nasal swabs from captive adult bighorns following pneumonia episode in November, 1987.

1987	1988	1989		1990
Nov-Dec	Aug-Nov	May-July	Oct-Nov	Apr
Infected Pen				
9/12 (75%)	7/15 (47%)	7/28 (25%)	3/16 (19%)	0/5 (0%)
Noninfected Pen				
0/5	0/12	0/7	0/12	0/5

Before the pneumonia episode, 16 lambs were born in 1986 and 1987 (Table 3). After the pneumonia episode in fall 1987, 11 lambs were born in 1988 and 1989. All these lambs died between 6-11 weeks after birth.

Table 3. Summary of bighorn sheep mortality in a captive herd which was affected by pneumonia in the fall of 1987.

1986	1987	1988	1989
6/7*	10/10	3/7	8/9
	3 ewes died "Pneumonia" Add adult ram December, 1987 Died November 1989	All lambs died "Pneumonia"	All lambs died "Pneumonia" Add 2 adult ewes January 13, 1989. Both died - July, November, 1989

*First number indicates lambs, second number indicates ewes.

In 1989 the mean age of death was day 61 (Table 4). One late lamb in 1989 was born August 14, after the other 7 lambs had died. In almost all cases, lambs were clinically healthy until 1-2 days before death, developed respiratory disease characterized by open mouth breathing and rapid respirations, and died the next day. At necropsy lesions were similar in all lambs and were consistent with acute fibrinohemorrhagic pneumonia with fibrinous pleuritis. Usually the cranial and cardiac lung lobes were involved. Pasteurella haemolytica was isolated from all lambs. Serotypes from 4 of the lambs included T_4, from 3 lambs, $T_{3,4}$, from 1 lamb, and $T_{3,4,10}$ from 1 lamb.

Table 4. Summary of bighorn lamb deaths in captivity, 1989, and isolates of Pasteurella haemolytica from these lambs.

Lamb No.	Age at death (days)	Isolation
1	47	P. haemolytica
2	53	P. haemolytica $T_{3,4,10}$
3	78	P. haemolytica T_4
4	60	P. haemolytica T_4
5	56	P. haemolytica T_4, $T_{3,4}$
6	70	P. haemolytica
7	78	P. haemolytica
8	43	P. haemolytica
	$\bar{x}$ = 61	

DISCUSSION

These studies support the importance of pneumonia in bighorn sheep and illustrate the short- and long-term effects of the disease. In experiment 1, the 2 bighorns exposed to domestic sheep developed respiratory diseases and died within 2 weeks of exposure to domestic sheep. Although the serotypes of P. haemolytica isolated from the domestic sheep and from the bighorns were different, it is likely that more serotypes were present in the domestic sheep than were isolated. Standard practice at Washington Animal Disease Diagnostic Laboratory is to isolate 1 or 2 morphologically similar bacterial colonies from each agar plate for characterization. If many serotypes are present, only 1 or 2 will be isolated. Tonsillar biopsies and tonsillar swabs from bighorn sheep yield a higher percentage of P. haemolytica isolates than do nasal swabs (Dunbar et al. 1990). Unfortunately, my data are from nasal swabs alone and represent active shedding of bacteria into the environment. Although stress may be important in predisposing bighorns to pneumonia, I feel that bacteria transfer from domestic sheep to bighorns with resultant pneumonia is an important management and biological concern. Previous experiments have demonstrated the adverse association between domestic sheep and bighorn sheep, indicating that serotypes A_2, T_3, T_4, and T_{10}, alone or in combination, are the most pathogenic known serotypes in bighorns (Onderka and Wishart 1988, Onderka et al. 1988, Foreyt 1989). Severe pneumonia in bighorns has also been reported to occur without the presence of domestic sheep (Onderka and Wishart 1984) and may be caused by similar serotypes of P. haemolytica.

In experiment 2, it is likely that P. haemolytica from domestic sheep initiated the pneumonia in the captive bighorn herd. Although the plastic tube used to lavage the domestic sheep was rinsed in saline and wiped with an alcohol swab, it is probable that some bacteria remained in the tube and infected the bighorns. All the bighorns developed chronic respiratory disease, and 3 died within 50 days. Under field conditions, a similar episode may have caused higher mortality because more energy would have been required for feeding and movement. Only 3 lambs were born to the 7 surviving ewes in 1988 due to their poor condition during the breeding season and early gestation. Seven ewes survived the pneumonia episode and became clinically normal, but many remained chronic shedders of P. haemolytica (Table 2) for over 2 years.

All lambs died of bronchopneumonia during 1988 and 1989 (Table 3). It is likely that lambs are protected against pneumonia resulting from P. haemolytica by colostral immunity. When the immunity wanes at 5-8 weeks after birth, P. haemolytica from their dams or other sheep overwhelm them, and cause the pneumonia and death observed in this experiment and probably in field outbreaks of pneumonia. Based on the reduced prevalence of ewes shedding P. haemolytica over time (Table 2), it is probable that lamb survival will increase in 1990.

The ram added to the affected herd of sheep in 1987, and the 2 ewes added in 1989 died of pneumonia between 7 months and 2 years later, indicating the long-term deleterious effects of P. haemolytica in adult, presumably noninfected bighorns.

Based on these and other studies (Onderka and Wishart 1984, Bailey 1986, Coggins 1988) it is probable that following a pneumonia episode in bighorn sheep, initial mortality will affect sheep of all ages, and subsequent mortality in future years will primarily affect lambs between 4-9 weeks old. Lamb survival may be correlated with prevalence of ewes that are shedding P. haemolytica in nasal secretions. Lamb mortality and mortality of healthy sheep introduced into affected populations are likely to be high for 2 or more years following a pneumonia outbreak. Breeding and lambing synchrony may be disrupted as indicated by late lambs and nonbreeding ewes. Based on these data, my recommendations for bighorn sheep management include: 1) exclusion of domestic sheep from contact with bighorn sheep by excluding domestic sheep from lands occupied by bighorns, and by preventing bighorn transplants into areas used by domestic sheep; 2) prohibition of moving healthy bighorns into populations of bighorns that have survived a pneumonia episode or are shedding P. haemolytica in nasal secretions; and 3) prohibition of moving survivors from pneumonia episodes, or bighorns shedding P. haemolytica in nasal secretions, into healthy populations. Considering the long-term recovery time of a population following a pneumonia die-off, a more drastic option is to kill all survivors, and then start a new population with healthy transplanted bighorns. However, a local gene pool would be lost. Until an effective vaccine is developed or a resistant strain of bighorn is discovered, preventive disease management is important to reduce the probability of pneumonia related mortality in bighorn sheep populations.

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