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EFFECTS OF CONTROLLED CONTACT EXPOSURE BETWEEN HEALTHY BIGHORN SHEEP AND LLAMAS, DOMESTIC GOATS, MOUNTAIN GOATS, CATTLE, DOMESTIC SHEEP, OR MOUFLON SHEEP

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Abstract: In separate experiments under controlled conditions, captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were placed on pasture with llamas (*Llama glama*), domestic goats, mountain goats (*Oreamnos americana*), cattle, and mouflon sheep (*Ovis musimon*) to determine the effects on the bighorn sheep. In an additional experiment, two domestic sheep and two bighorn sheep were placed together in an isolation facility. Essentially all bighorn sheep, domestic goats, domestic sheep, cattle, mountain goats, and mouflon sheep were pharyngeal carriers of *Pasteurella haemolytica* when the contact experiments began. The most common serotype of *P. haemolytica* reacted in antisera to T3, 4,and 10. *Pasteurella haemolytica* was not isolated from the three llamas used in these experiments nor from 14 additional llamas sampled. Bighorn sheep remained clinically healthy during and after contact with llamas, cattle, mountain goats, and domestic goats, but all bighorn sheep died from acute bronchopneumonia after contact with domestic sheep and mouflon sheep.

Respiratory disease caused by *Pasteurella haemolytica* in Rocky Mountain bighorn sheep is the most important disease affecting their survival in North America. Catastrophic mortality and poor lamb survival from surviving ewes are the two major characteristics associated with these pneumonia episodes in bighorn sheep (Onderka and Wishart 1984, Coggins 1988, Foreyt 1989, 1990). Previous research indicates that bighorn sheep are highly susceptible to respiratory disease (Silflow et al. 1991, 1993), and a variety of factors including lungworms, viruses, bacteria, and stress components can be important in the respiratory disease complex (Spraker et al. 1984, Foreyt 1990).

Experimental contact studies between domestic sheep and bighorn sheep under captive conditions resulted in significant mortality due to pneumonia in the bighorn sheep, and no mortality or respiratory disease in the domestic sheep (Foreyt 1989, 1990, 1992a). *Pasteurella haemolytica*, predominantly biotype A, serotype 2 (A2) was the usual organism isolated from the lungs of the dead bighorn sheep. *Pasteurella haemolytica* A2 is a common organism carried in the pharyngeal area of domestic sheep (Thompson et al. 1977, Frank 1982), but rarely is isolated from healthy bighorn sheep (Dunbar et al. 1990). Experimentally, *P. haemolytica* A2 from healthy domestic sheep inoculated intratracheally into bighorn sheep and healthy domestic sheep, resulted in acute fatal pneumonia in 7 of 8 of the inoculated bighorn sheep, whereas the domestic sheep and non- contact bighorn sheep remained healthy (Foreyt et al. 1994). The inoculum strain of *P. haemolytica* A2 was evaluated by a genomic fingerprinting technique known as ribotyping (Snipes et al. 1992), and the ribotype in the inoculum was the same ribotype recovered from all the dead bighorns. This experiment indicated that some strains of *P. haemolytica* from healthy domestic sheep are lethal in bighorn sheep. Based on all published data, contact between domestic sheep and bighorn sheep must be avoided to prevent the mortality associated with those strains of *P. haemolytica* in domestic sheep that are lethal in bighorn sheep.

The purpose of these studies was to determine the compatibility of bighorn sheep with other ungulates that may potentially have close contact with bighorn sheep in wild or captive situations.

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MATERIALS AND METHODS

Six experiments were done with Rocky Mountain bighorn sheep at Washington State University, Pullman, Washington, by placing other ungulates with them on common pasture to determine whether the animals were compatible for disease transmission and survival. All animals were grazed together on common pasture for 60 days, unless specified, and were clinically healthy at the initiation of each experiment.

Microbiology Techniques

At the beginning and end of each experiment, pharyngeal swab samples were collected from all animals for bacterial isolations. A harp speculum was used to hold the mouth open and restrain the tongue. After the pharyngeal area was observed, a sterile polyester-tipped applicator swab (Spectrum Laboratories, Inc., Houston, Texas, USA) was used to rub the pharyngeal area briskly, removed, and transported to WADDL, Pullman, Washington. All swabs were streaked onto 5% sheep blood agar plates within 2 hr of collection to maximize isolation of *P. haemolytica* (Wild and Miller, 1991).

Isolation and identification of *P. haemolytica* was accomplished by the methods of Snipes et al. (I992) , but hemolysis on 5% sheep blood agar or growth on MacConkey's agar were not requisites for identification (Onderka et al. 1988; Wild and Miller I991). All *P. haemolytica* isolates were identified to serotype by rapid plate agglutination (Frank and Wessman 1978). If an isolate cross-reacted between or among serotypes, all were listed.

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 I992). 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 all animals were evaluated for lungworm larvae by a modified Baermann technique (Beane and Hobbs, I983).

Experiment 1 -Bighorn Sheep and Mouflon Sheep.

Six bighorn sheep and 5 mouflon sheep (Table 1) were placed together in a 0.4 ha pen which contained various grasses, and a shelter. Trace mineral salt, alfalfa hay, alfalfa pellets and water were available at all times. The bighorn sheep had been in captivity at Washington State University for approximately one year and consisted of ewes and rams ranging in age from 1 to 3 years. The mouflon sheep were obtained from a private game farm and were rams and ewes ranging in age from 1 to 7 years.

Experiment 2 - Bighorn Sheep and Domestic Goats.

Two bighorn sheep and 3 domestic goats were placed together in the same pen and held under the same conditions as described in experiment 1. The bighorn sheep yearling rams had been in captivity all of their lives while the wether yearling goats were purchased from a local livestock auction.

Experiment 3 - Bighorn Sheep and Mountain Goats.

Nine bighorn sheep and 2 mountain goats were placed together in a 0.8 ha pen which contained various grasses, pine trees and a shelter. Trace mineral salt, alfalfa hay, alfalfa pellets and fresh water were available at all times. The bighorn sheep composed a breeding herd that had been in captivity for approximately 6 years and included a 6 yr-old ram, a 2-yr old ram, and 7 adult ewes. The mountain goats, obtained from a commercial zoo, were 5 mo-old male kids.

Experiment 4 - Bighorn Sheep and Llamas.

Subsequent to experiment 3, the same nine bighorn sheep were placed together with 3 llamas and maintained in the same manner and pen described in experiment 3. The llamas were geldings between 1 and 4 years old that had been donated to Washington State University. A total of 17 llamas were initially sampled in an attempt to find 3 that were carriers of *P. haemolytica*. None was found; therefore, 3 easily accessible llamas were chosen.

Experiment 5 - Bighorn Sheep and Cattle.

Four bighorn sheep and 3 cattle were placed together in the pen described in experiment 1. The bighorn sheep were 8 mo-old lambs (2 males and

2 females) that were obtained from Wildhorse Island on Flathead Lake in Montana one month prior to the experiment. The cattle were Holstein steers that weighed approximately 200 kg each.

Experiment 6 - Bighorn Sheep and Domestic Sheep.

Two yearling bighorn sheep rams and 2 castrated yearling domestic sheep were placed together in an indoor isolation facility 4 x 7 m. Trace mineral salt, alfalfa hay, hay pellets, and fresh water were available at all times.

Evaluation

All animals were observed at least twice daily for signs of respiratory disease. If animals developed respiratory disease, they were euthanized with an intravenous injection of sodium pentobarbital. All dead sheep were submitted to WADDL for complete necropsy evaluation. Bacterial isolations on blood agar were attempted from tissues including tonsil, liver, 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 animals approximately 60 days after the animals were placed together.

RESULTS

At the initiation of each experiment, *P. haemolytica* was isolated from all animals except the 3 llamas and 1 bighorn sheep (Tables 1 - 6). The most common isolate of *P. haemolytica* reacted in antisera to T3,4,and 10. Viruses were not isolated from any animal, and lungworm larvae were detected in low numbers (<10 per gram of feces) in approximately half of the bighorn sheep.

All animals survived and remained healthy (Tables 1 - 6) except the bighorn sheep in contact with mouflon sheep (experiment 1) or domestic sheep (experiment 6). All 5 bighorn sheep died on days 41 or 42 after initial contact with the mouflon sheep (Table 1), and the 2 bighorn sheep died on days 6 and 8, respectively, after initial contact with domestic sheep (Table 6). At necropsy, all bighorn sheep were in good body condition with adequate amounts of body fat. Lesions were similar in the 7 bighorn sheep that died and were characteristic of acute, fibrino-hemorrhagic pneumonia and pleuritis. Up to 80% of lung volume was dark red and consolidated with moderate amounts of adherent fibrin. On cut surface, lungs were diffusely edematous with prominent interlobular septa. Regional lymph nodes (mandibular, cervical, tracheobronchial, mediastinal) were moderately 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 and 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.

The primary biotype and serotype of *P. haemolytica* recovered from tissues of dead bighorns was A2, which had not been recovered from the bighorn sheep at the initiation of the experiment. None of the isolates recovered from bighorn sheep at the initiation of the experiments were toxic as determined by the neutrophil sensitivity test (Silflow et al. 1990), but all biotype A isolates recovered from dead bighorn sheep after contact with mouflon sheep were toxic (Table 1). A toxic biotype A untypeable serotype of *P. haemolytica* was recovered from one of the mouflon sheep initially, but no toxic isolates were recovered from mouflon sheep at the termination of the experiment (Table 1). Toxicities were not evaluated from domestic sheep and bighorn sheep in experiment 6, but *P. haemolytica* A2 was detected in both domestic sheep at the initiation of the experiment and in both dead bighorns at necropsy (Table 6). Toxic isolates of *P. haemolytica* were not detected in the domestic goats, mountain goats, llamas, or cattle (Tables 2 - 5).

DISCUSSION

As indicated in these experiments, *P. haemolytica* is detected commonly from a variety of healthy ungulates including bighorn sheep, cattle, domestic goats, mouflon sheep, and domestic sheep. Pneumonia in bighorn sheep caused by *P. haemolytica* can occur with or without contact with other ungulates (Miller et al. 1991) and it is now

clear that some serotypes or strains of *P. haemolytica* carried by some animals are likely to result in fatal pneumonia in bighorn sheep (Foreyt et al. 1994). The current results support previously published research which documented the incompatibility between domestic sheep and bighorn sheep (Onderka and Wishart 1988; Foreyt 1989, 1990, 1992a). Based on current results and previous findings (Callan et al. 1991), close contact between mouflon sheep and bighorn sheep also is likely to result in fatal pneumonia in the bighorn sheep.

Contact experiments between bighorn sheep, domestic goats, llamas, cattle, and mountain goats did not result in respiratory disease or death of any of the animals. Based on our experience with bighorn sheep, *P. haemolytica* A2 is the most serious pathogen of bighorn sheep. Toxicity studies now in progress in our laboratory, indicate that the A biotype of *P. haemolytica*, primarily serotype 2, frequently is toxic to blood neutrophils in vitro and to bighorn sheep in vivo, whereas the T biotype usually is nontoxic to blood neutrophils and to bighorn sheep. Only isolates of *P. haemolytica* biotype T were detected in the cattle and domestic goats used in these experiments, therefore, to fully understand the compatibility status of these animals, similar work should be repeated using cattle and goats that are known carriers of the A biotype.

MANAGEMENT RECOMMENDATIONS

As a result of these and previous studies, specific management recommendations can be made. All contact between bighorn sheep and domestic sheep or mouflon sheep must be prevented or it is likely that the bighorn sheep will die from pneumonia after close contact with these species. Based on available data, bighorn sheep contact with elk (*Cervus elaphus*), deer (*Odocoileus virginianus* and *O. hemionus hemionus*), mountain goats, or llamas apparently does not result in respiratory disease in bighorn sheep (Foreyt 1992b, this study). Trials with domestic goats and cattle did not result in respiratory disease in bighorn sheep under the conditions described in this experiment. However, similar trials need to be conducted with domestic goats and cattle that are carriers of *P. haemolytica* biotype A to determine the effects of those organisms on the health of bighorn sheep.

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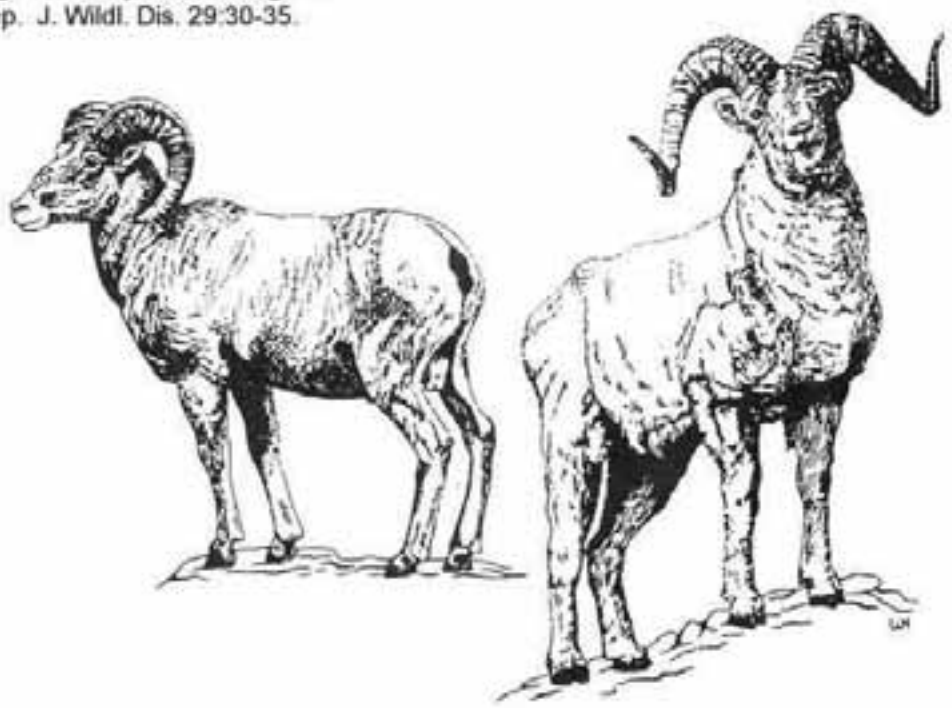

Table 1. *Pasteurella haemolytica* biotypes and serotypes isolated from bighorn sheep and mouflon sheep that shared the same pasture.

Animal	Pre-exposure isolates (day 0)	Cytotoxic[a]	Post-exposure isolates[b]	Cytotoxic[a]	Pneumonia	Day of death
Bighorn sheep 1	T3,4	-	A2 (lung)	+	+	42
			T3,4,10(lung)	+		
			A2(liver)	+		
Bighorn sheep 2	T3,4,10	-	A unt[c] (lung)	+	+	42
			T3,4 (lung)	-		
Bighorn sheep 3	T3,4	-	A2 (lung)	+	+	42
			A unt(lung)	+		
			T unt (lung)	-		
Bighorn sheep 4	T3,4,10	-	A2 (lung)	+	+	42
			T3,4,10 (lung)	-		
Bighorn sheep 5	T3,4,10	-	A2 (lung)	+	+	41
Bighorn sheep 6	T3,4,10	-	A2 (lung)	+	+	41
Mouflon sheep 1	T unt	-	T3,4	-	-	NA[d]
			T unt	-		
Mouflon sheep 2	A (unt)	+	T3,4	-	-	NA
			A unt	-		
Mouflon sheep 3	T4	-	T unt	-	-	NA
Mouflon sheep 4	T4	-	T3,4	-	-	NA
Mouflon sheep 5	T4	-	T3,4	-	-	NA
			A unt	-	-	

[a] Based on neutrophil sensitivity test (Silflow et al. 1993).
[b] At necropsy or 47 days after initial contact.
[c] unt = untypeable
[d] NA = not applicable.

Table 2. *Pasteurella haemolytica* biotypes and serotypes isolated from bighorn sheep and domestic goats that shared the same pasture for 60 days.

Animal	Pre-exposure isolates (day 0)	Cytotoxic[a]	Post-exposure isolates (day 60)	Cytotoxic[a]	Pneumonia
Bighorn sheep 7	T3,4,10	-	T3,4,10	-	-
Bighorn sheep 8	T3,4,10	-	T3,4,10	-	-
Domestic goat	T unt[b]	-	T3,4	-	-
Domestic goat 2	T3,4	-	T3,4	-	-
Domestic goat 3	T unt	-	T3,4	-	-

[a] Based on neutrophil sensitivity test (Silflow et al. 1993) [b] unt = untypeable

Table 3. *Pasteurella haemolytica* biotypes and serotypes isolated from bighorn sheep and mountain goats that shared the same pasture for 60 days.

Animal	Pre-exposure isolates (day 0)	Cytotoxic[a]	Post-exposure isolates (day 60)	Cytotoxic[a]	Pneumonia
Bighorn sheep 9	T untb	-	T4	-	-
Bighorn sheep 10	T unt	-	T3,4	-	-
Bighorn sheep 11	unt	-	T3,4,10	-	-
Bighorn sheep 12	T3,4,10	-	T3,4,10	-	-
	T unt	-			
Bighorn sheep 13	T unt	-	T unt	-	-
Bighorn sheep 14	T3,4	-	T3,4	-	-
Bighorn sheep 15	T unt	-	T3,4,10	-	-
Bighorn sheep 16	T4	-	T4	-	-
Mountain goat 1	T3,4	-	ND[c]	NA[d]	-
Mountain goat 2	T3,4	-	ND	NA	-

[a] Based on neutrophil sensitivity test (Silflow et al. 1993).
[b] unt = untypeable.
[c] ND = not done.
[d] NA = not applicable

Table 4. *Pasteurella haemolytica* biotypes and serotypes isolated from bighorn sheep and llamas that shared the same pasture for 68 days.

Animal	Pre-exposure isolates (day 0)	Cytotoxic[a]	Post-exposure isolates (day 68)	Cytotoxic[a]	Pneumonia
Bighorn sheep 9	T3	-	T3,4	-	-
Bighorn sheep 10	none	NA[b]	T3,4,10	-	-
Bighorn sheep 11	T3,4	-	T3,4,10	-	-
	T3,4,10	-			
Bighorn sheep 12	T3,4,10	-	T3,4	-	-
	T unt[c]	-	T unt	-	-
Bighorn sheep 13	T3,4	-	T3,4,10	-	-
Bighorn sheep 14	T3,4,10	-	T3,4,10	-	-
Bighorn sheep 15	none	NA	T3,4	-	-
Bighorn sheep 16	T3,4,10	-	T unt	-	-
Llama 1	none	NA	none	NA	-
Llama 2	none	NA	none	NA	-
Llama 3	none	NA	none	NA	-

[a] Based on neutrophil sensitivity test (Silflow et al. 1993). [b] NA = not applicable. [c] unt = untypeable.

Table 5. ***Pasteurella haemolytica*** **biotypes and serotypes isolated from bighorn sheep and cattle that shared the same pasture for 60 days.**

Animal	Pre-exposure isolates (day 0)	Cytotoxic[a]	Post-exposure isolates (day 68)	Cytotoxic[a]	Pneumonia
Bighorn sheep 17	T3,4,10	-	T3,4,10	-	-
	T4	-			
Bighorn sheep 18	T3,4	-	T3,4	-	-
	T4	-			
Bighorn sheep 19	T3,4	-	T3,4	-	-
	T3,4,10	-			
Bighorn sheep 20	T4	-	T3,4	-	-
	T3,4,10	-			
Calf 1	T3,4	-	none	Na[b]	-
Calf 2	T3,4	-	T3,4	-	-
Calf 3	T3,4	-	none	NA	-

[a] Based on neutrophil sensitivity test (Silflow et al. 1993).
[b] NA = not applicable.

Table 6. ***Pasteurella haemolytica*** **biotypes and serotypes isolated from bighorn sheep and domestic sheep the shared the same isolation facility.**

Animal	Pre-exposure isolates (day 0)	Post-exposure isolates[a]	Pneumonia	Day of death
Bighorn sheep 1	T3	A2 (liver)	+	6
		A2 (lung)		
Bighorn sheep 2	T3	A2 (lung)	+	8
		T3 (lung)		
Domestic sheep 1	T3,4,10	T3,4	-	NA[b]
	A2			
Domestic sheep 2	A2	T3,4	-	NA

[a] At necropsy or 14 days after initial contact.
[b] NA = not applicable.