EVALUATION OF A MULTIVALENT *PASTEURELLA HAEMOLYTICA* TOXOID-BACTERIN IN PROTECTING BIGHORN SHEEP FROM PNEUMONIA AFTER EXPOSURE TO DOMESTIC SHEEP

BILL FOREYT, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164, USA

Abstract: A *Pasteurella haemolytica* toxoid-bacterin which contained serotypes A1, A2, and T10 was evaluated in captive bighorn sheep to determine efficacy in protecting bighorn sheep from pneumonia after exposure to domestic sheep. Six healthy captive bighorn sheep were divided randomly into two equal groups and then balanced for age. Three bighorn sheep were each vaccinated intramuscularly twice with 2 mls of vaccine on day minus 28 and on day minus 14 of the experiment. Three unvaccinated bighorn sheep were each given an intramuscular injection of two mls of sterile water on the same days the other three bighorns were vaccinated. On day 0, three healthy domestic sheep were placed in the pen with the bighorn sheep and maintained with the bighorns for 60 days. All six bighorn sheep died from clinical pneumonia during the experiment, and all three domestic sheep remained healthy. Vaccinated bighorns died on experimental days 30, 30, and 61; unvaccinated bighorn sheep died on experimental days 20, 30, and 32. *Pasteurella* spp isolated from lungs or tissues of dead bighorn sheep included several serotypes of *P. haemolytica* and *P. multocida*. Cytotoxin neutralizing antibodies and agglutinating antibodies against A1, A2, and T10 were present in all bighorn sheep before vaccination. Antibody titers did not differ between vaccinated and unvaccinated bighorn sheep, and were not elevated after vaccination. This experiment indicated that the vaccine did not protect bighorn sheep against pneumonia after exposure to domestic sheep.

Pasteurellosis, caused by *Pasteurella haemolytica* and *Pasteurella multocida*, remains the major mortality factor in free-ranging bighorn sheep (*Ovis canadensis*) populations (Foreyt, 1990; Cassirer et al., 1996). Outbreaks of pasteurellosis in bighorn sheep often results in widespread mortality initially, followed by very low recruitment for several years following the die-off (Foreyt, 1990). Although some strains of *Pasteurella* spp. carried by domestic sheep are directly lethal to bighorn sheep (Foreyt et al., 1994), other factors potentially can predispose to or exacerbate mortality. Such factors include other bacteria, respiratory viruses, other microbial agents, lungworms, gastrointestinal and external parasites, genetics, increased population density, habitat, nutrition, adverse weather, presence of domestic livestock and other wildlife, breeding activity, behavior, capture and restraint techniques, and other stressors. Management methods that have been suggested to prevent pasteurellosis in bighorn sheep include preventing contact with domestic or mouflon sheep (*Ovis musimon*), reducing the factors mentioned previously that can contribute to pneumonia outbreaks, and vaccinating bighorns with a safe and effective vaccine.

Currently, the are no vaccines that have been shown to be effective against the lethal strains of *P. haemolytica*, primarily biotype A, serotype 2, carried by domestic sheep. Previous vaccine studies with captive bighorn sheep exposed to domestic sheep included a commercially available bacterin (Foreyt, 1989), and an autogenous bacterin (Foreyt, 1992). In those experiments, all vaccinated and unvaccinated bighorns died from bronchopneumonia. One additional vaccine study that evaluated a live nonlethal cytotoxic *P. haemolytica* A2 was not successful in preventing pneumonia when vaccinated bighorns were inoculated with cytotoxic *P. haemolytica* A2 from domestic sheep (Foreyt and Silflow, 1996). Recently Miller et al., 1997, and Kraabel et al., 1998, evaluated a multivalent *P. haemolytica* vaccine, containing A1, A2, and T10, in bighorn sheep to determine safety, serologic responses and protection from experimental challenge with a bighorn sheep strain of *P. haemolytica* T10. Results from those experiments indicated the vaccine was safe, increased leukotoxin neutralizing titers and agglutination titers to A1 and A2, and resulted in lower morbidity and mortality in vaccinated bighorn sheep during the 7 days after inoculation.

The purpose of the current experiment was to determine if this multivalent *P. haemolytica* vaccine would protect bighorn sheep against pneumonia after exposure to domestic sheep.

I thank M. Miller for providing the vaccine, H. McNeil for serological analysis, R. Rimler for *P. multocida* analysis, J. Lagerquist for serotyping and parasite analysis, and the personnel in the Washington Animal Disease Diagnostic Laboratory for bacterial and viral evaluations. Partial funding from the Foundation for North American Wild Sheep is appreciated.

MATERIALS AND METHODS

Six captive Rocky Mountain bighorn sheep and three domestic sheep were used in this experiment. The bighorn sheep were divided randomly into two equal groups and then balanced for age and sex. The vaccinated group consisted of two 1-yr-old males and a 5-yr-old male. The unvaccinated control group consisted of a 1 yr-old male, a 1yr-old female, and a 3-yr-old male. The vaccinated sheep were each given an intramuscular injection of 2-mls of an experimental *P. haemolytica* bacterin-toxoid vaccine that contained A1, A2, and T10 on days minus 28 and minus 14 before domestic sheep exposure. Dr. Michael Miller from the Colorado Division of Wildlife kindly supplied the vaccine. Unvaccinated control bighorn sheep were each given an intramuscular injection of 2-mls of sterile water. All injections were in the gluteal muscles of the hind leg.

Four weeks after administration of the first vaccine and two weeks after the second vaccine, three clinically healthy, adult, male domestic sheep were moved into the pen with the six bighorn sheep. The domestic sheep were obtained from the University of Idaho Sheep Center, Moscow Idaho. Pharyngeal swab samples for bacterial analysis were collected from all bighorn sheep on days of vaccination (days –28 and –14), and the day when domestic sheep were introduced (day 0). Pharyngeal swab samples were collected from all domestic sheep on days 0 and 30. Domestic sheep maintained residence in the pen with the bighorn sheep for 60 days, at which time they were removed.

Pharyngeal swab samples from all animals were placed in Amies transport medium (Spectrum Diagnostics, Inc., Houston, Texas, 77032, USA), transported to the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington, 99164, USA, and streaked onto 5% sheep blood agar within 2 hr of collection to maximize isolation of *P. haemolytica* (Wild and Miller, 1991). Isolation and identification of *P. haemolytica* and *P. multocida* was accomplished by standard methods (Carter, 1984, Snipes et al., 1992), but hemolysis on 5% sheep blood agar or growth on MacConkey's agar were not requisites for identification of *P. haemolytica* (Onderka et al., 1988). All *P. haemolytica* isolates were identified to biotype and serotype according to established formats (Biberstein, 1978; Frank and Wessman, 1978). When an isolate reacted in antisera to several serotypes, all were listed. Isolates of *P. multocida* were characterized by Dr. Richard Rimler, National Animal Disease Center, Ames, Iowa, according to previously established methods (Rimler and Brodgden, 1986). Capsule group was determined by the mucopolysaccharidase test using chrondroitinase AC, hyaluronidase, and heparinase III. Somatic type was determined by the gel-diffusion precipitin test, and toxin production was determined with monoclonal anti-PMDT in a colony lift assay.

At the beginning of the experiment, nasal swab samples (Marion Scientific Viral Culturette, Marion Scientific, Kansas City, Kansas 69114, USA) also were collected for virus evaluation. Specimens were inoculated onto ovine fetal tracheal cells and bovine turbinate cells for 2 passages at ten-day intervals and were examined daily for cytopathic effect. Additional specimens were tested for respiratory syncytial virus by use of solid phase-enzyme immunoassay (Abbott RSV EIA, Abbott Laboratories, South Pasadena, California 91030, USA). Isolation of *Chlamydia* sp.was not attempted, and fecal samples were evaluated for lungworm larvae by a modified Baermann technique (Foreyt, 1997). A serological screen for antibodies to respiratory viruses, malignant catarrhal fever virus, and *Brucella ovis* was done by WADDL according to established procedures.

Evaluation

For the entire experiment, all sheep were observed at least once daily for signs of respiratory disease. Sheep that died were submitted to WADDL for complete necropsy evaluation. At necropsy, bacterial isolations were attempted from several tissues including tonsil, bronchial lymph nodes, spleen and lungs. Representative tissues also were fixed in 10% buffered formalin, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic evaluation.

Pasteurella serology

Measurement of leukotoxin neutralizing antibodies and agglutinating antibodies to serotype-specific surface antigens was done by Heather McNeil, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, according to the methods published by Miller et al., 1997. Titers were expressed as $\log_2$. For comparative purposes, additional serum samples were collected from 12 adult, healthy, free-ranging bighorn sheep (5 rams and 7 ewes) at Hall Mountain in northeastern Washington (Foreyt et al., 1996).

RESULTS

All six bighorn sheep died from clinical pneumonia between 20 and 61 days after initial exposure to domestic sheep (Table 1). Vaccinated bighorns died on days 30, 30, and 61, respectively, and unvaccinated bighorns died on days 20, 30, and 32, respectively.

Table 1. Identification, age, sex, and day of death of bighorn sheep used on the vaccination experiment.

	Age	Sex	Day of Death*
Vaccinated Bighorns			
#1 (Tag 44)	1yr	M	30
#2 (Tag 43)	1 yr	M	30
#3 (Tag 146)	5 yr	M	61
Unvaccinated Bighorns			
#4 (Tag 46)	1 yr	F	20
#5 (Tag 22)	3 yr	M	30
#6 (Tag 47)	1 yr	M	32

* Days after exposure to domestic 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 75% 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. Tapeworms, *Wyominia tetoni* were present in the hepatic bile ducts of two of the sheep.

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.

Pasteurella spp. were isolated from pharyngeal swab samples from all six bighorn sheep before exposure to domestic sheep and from two of three of the domestic sheep (Table 2). Most isolates were *P. haemolytica*, biotype T (also called *Pasteurella trehelosi*). *Pasteurella* spp., including *P. multocida* and several serotypes of *P. haemolytica* were isolated from the lungs, lymph nodes or other tissues of all six dead bighorns at necropsy (Table 2). Important *Pasteurella* spp. included *P. haemolytica* A2, which was isolated from two dead bighorn sheep, and P. *multocida* D:3 which was isolated from three dead bighorn sheep.

All domestic sheep remained healthy throughout the experiment. On days 0 and 30, several serotypes of *P. haemolytica* were isolated from two of the domestic sheep, but no *Pasteurella* spp. were isolated from the third sheep. *Pasteurella multocida* was not isolated from any of the domestic sheep (Table 2).

Table 2. Summary of *Pasteurella haemolytica* and *Pasteurella multocida* isolated during the vaccination experiment.

Group	Day B28, -14, or 0[a]	At Necropsy
Vaccinated		
Bighorn 1	*P. haemolytica* $T_{3,4}$; Tunt[b]; $A_{2,5,6}$; A_5	*P. haemolytica* A_5, $A_{2,5}$
Bighorn 2	*P. haemolytica*, $T_{3,4}$; Tunt; $A_{2,5}$	*P. multocida* D:3
Bighorn 3	*P. haemolytica* Tunt, unt	*P. haemolytica* unt, *P. multocida* D:3
Unvaccinated		
Bighorn 4	*P. haemolytica*, $T_{3,4,10,15}$; Tunt; $A_{2,5,6,8,11}$; Aunt	*P. haemolytica* A_5; Aunt
Bighorn 5	*P. haemolytica*, $T_{3,4}$; $T_{3,4,10,15}$	*P. haemolytica* unt
Bighorn 6	*P. haemolytica*, $T_{3,4,15}$; Tunt; $_{2,5,6}$; $A_{1,8,11}$; Aunt	*P. multocida* D:3
Domestic Sheep	Day 0	Day 30
1	*P. haemolytica* T_4	*P. haemolytica*, $T_{3,4,10,15}$
2	*P. haemolytica* $T_{3,4}$ *P. haemolytica* T_4	*P. haemolytica* unt
3	negative	negative

[a] Days B28 and B14 are the days of vaccination; day 0 is the day of domestic sheep introduction
[b] Untypeable

No viruses or lungworm larvae were isolated from any of the bighorn sheep. Antibody titers were not detected against parainfluenza 3 virus, bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus, bovine virus diarrhea virus, ovine progressive pneumonia virus *or Brucella ovis*. Antibodies against malignant catarrhal fever virus were detected in bighorn sheep numbers 3, 4, and 6.

Leukotoxin neutralizing antibodies, and agglutinating antibodies against surface antigens of A1, A2, and T10 were detected in all bighorn sheep on days -28, -14, and 0 (Table 3). Antibody titers were similar between vaccinated and unvaccinated bighorns, and were not elevated after vaccination. Antibody titers from 12 healthy, adult bighorn sheep from the Hall Mountain herd in northeastern Washington are listed in Table 4. Bighorn sheep tested from the Hall Mountain herd had antibody titers that were similar to the titers of the experimental bighorn sheep.

DISCUSSION

The purpose of this experiment was to determine whether vaccination of bighorn sheep with this multivalent vaccine would protect bighorn sheep against fatal pneumonia that predictably occurs after exposure to domestic sheep (Foreyt, 1989; Martin et al., 1996). Because all bighorn sheep died of pneumonia during this experiment, my conclusion is that the vaccine did not protect the bighorn sheep against pneumonia. *Pasteurella haemolytica* A2, which is one bacteria that is

carried by a high percentage of healthy domestic sheep and has been shown to be lethal in bighorn sheep (Foreyt, 1989), was isolated at necropsy from one vaccinated and one unvaccinated bighorn sheep. This serotype was not isolated from the three domestic sheep or any bighorn sheep before death; therefore, the source of the bacteria is unknown. However, based on previous experience and data that indicate a major percentage of domestic sheep are carriers of A2, it may be that one or more of these three domestic sheep were sporadic shedders of A2 and it was not detected at the time of sampling, or it was present in very low numbers in the domestic sheep, and was not detected by laboratory personnel.

Pasteurella multocida was not isolated from any live bighorn or domestic sheep from pharyngeal swab samples, but was isolated at necropsy from tissues of three of the dead bighorn sheep. The source of the *P. multocida* could not be determined.

All bighorns had relatively high antibody titers to the components in the vaccine before they were vaccinated, and vaccination did not significantly increase those titers. These data are in contrast to those of Miller et al., 1997, and Kraabel et al., 1998, who evaluated this vaccine for safety, serological responses to the vaccine, and protection from experimental challenge with *P. haemolytica* T10. Miller et al., 1997, reported mean pretreatment titers for *P. haemolytica* leukotoxin neutralizing antibody of less than 1; whereas, in the current experiment, bighorn sheep titers ranged from 2 to 10 (mean of 6.3), and in the healthy free ranging bighorn sheep, titers ranged from 2-7 (mean of 4.8). It may be that many of these titers are cross reactions with nonspecific antigens, and may not accurately reflect exposure to the specific serotypes of *P. haemolytica*. They also reported marked increase in antibody titers to A1 and A2 surface antigens after vaccination. In the current experiment the change from pre to post vaccination titers was negligible.

Although the vaccine did not protect the bighorn sheep from pneumonia after exposure to domestic sheep in this experiment, some of the deaths may have been caused by *P. multocida*, which was not one of the components of the vaccine. It follows that future vaccine research should involve vaccines that contain *P. multocida* as one component. Because *P. haemolytica* A2 is common in domestic sheep and highly pathogenic in bighorn sheep, it is essential that an effective vaccine contain the A2 component to protect bighorn sheep if they are likely to be exposed to domestic sheep. Additional work on *P. multocida* and vaccine evaluation in bighorn sheep is justified because of the severe

Table 3. Antibody titers (log_2) on days B28, -14 and 0 of the vaccine experiment.

	Vaccinated Bighorn Sheep				Unvaccinated Bighorn Sheep			
Pasteurella haemolytica antibody	A_1	A_2	T_{10}	LNA[a]	A_1	A_2	T_{10}	LNA[a]
Bighorn sheep number	1,2,3	1,2,3	1,2,3	1,2,3	4,5,6	4,5,6	4,5,6	4,5,6
Day B 28 (1st vaccination)	6,6,6	9,7,14	8,8,8	8,6,10	4,8,5	8,8,9	8,9,8	2,2,10
	(5.7)[b]	(10.0)	(8.0)	(8.0)	(5.7)	(8.3)	(8.3)	(4.7)
Day B14 (2nd vaccination)	6,5,11	8,7,15	7,7,9	7,5,11	7,7,5	8,7,9	9,9,8	1,6,10
	(7.3)	(10.0)	(7.7)	(7.7)	(5.7)	(8.0)	(8.7)	(5.7)
Day 0 (Domestic sheep introduced)	5,6,12	7,8,16	8,7,9	3,7,12	5,6,4	7,7,9	8,9,9	0,5,5,11
	(7.7)	(10.3)	(8.0)	(7.3)	(4.7)	(7.7)	(8.7)	(5.5)

[a] LNA = leukotoxin neutralizing antibody titer

[b] Number in parenthesis is the mean titer of the three bighorns

Table 4. *Pasteurella haemolytica* antibody titers (log_2) from adult free-ranging bighorn sheep (n = 12) from a healthy herd in Northeastern Washington.

Antibody	Range of titers	Mean titer	Median titer
A_1[a]	3-6	4.3	4
A_2[a]	5-7	6.3	6
T_{10}[a]	7->12	9.3	9
LNA[b]	2-7	4.8	5

[a] Agglutinating antibody titers (log_2)

[b] Leukotoxin neutralizing antibody titers (log_2)

manifestations of pasteurellosis in bighorn sheep populations and management goals. Use of a safe and effective vaccine against pathogenic strains of *Pasteurella* in bighorn sheep would be a valuable management technique, especially when bighorn sheep are captured or transplanted to new areas.

DISCUSSION

The purpose of this experiment was to determine whether vaccination of bighorn sheep with this multivalent vaccine would protect bighorn sheep against fatal pneumonia that predictably occurs after exposure to domestic sheep (Foreyt, 1989; Martin et al., 1996). Because all bighorn sheep died of pneumonia during this experiment, my conclusion is that the vaccine did not protect the bighorn sheep against pneumonia. *Pasteurella haemolytica* A2, which is one bacteria that is carried by a high percentage of healthy domestic sheep and has been shown to be lethal in bighorn sheep (Foreyt, 1989), was isolated at necropsy from one vaccinated and one unvaccinated bighorn sheep. This serotype was not isolated from the three domestic sheep or any bighorn sheep before death; therefore, the source of the bacteria is unknown. However, based on previous experience and data that indicate a major percentage of domestic sheep are carriers of A2, it may be that one or more of these three domestic sheep were sporadic shedders of A2 and it was not detected at the time of sampling, or it was present in very low numbers in the domestic sheep, and was not detected by laboratory personnel.

Pasteurella multocida was not isolated from any live bighorn or domestic sheep from pharyngeal swab samples, but was isolated at necropsy from tissues of three of the dead bighorn sheep. The source of the *P. multocida* could not be determined.

All bighorns had relatively high antibody titers to the components in the vaccine before they were vaccinated, and vaccination did not significantly increase those titers. These data are in contrast to those of Miller et al., 1997, and Kraabel et al., 1998, who evaluated this vaccine for safety, serological responses to the vaccine, and protection from experimental challenge with *P. haemolytica* T10. Miller et al., 1997, reported mean pretreatment titers for *P. haemolytica* leukotoxin neutralizing antibody of less than 1; whereas, in the current experiment, bighorn sheep titers ranged from 2 to 10 (mean of 6.3), and in the healthy free ranging bighorn sheep, titers ranged from 2-7 (mean of 4.8). It may be that many of these titers are cross reactions with nonspecific antigens, and may not accurately reflect exposure to the specific serotypes of *P. haemolytica*. They also reported marked increase in antibody titers to A1 and A2 surface antigens after vaccination. In the current experiment the change from pre to post vaccination titers was negligible.

Although the vaccine did not protect the bighorn sheep from pneumonia after exposure to domestic sheep in this experiment, some of the deaths may have been caused by *P. multocida*, which was not one of the components of the vaccine. It follows that future vaccine research should involve vaccines that contain *P. multocida* as one component. Because *P. haemolytica* A2 is common in domestic sheep and highly pathogenic in bighorn sheep, it is essential that an effective vaccine contain the A2 component to protect bighorn sheep if they are likely to be exposed to domestic sheep. Additional work on *P. multocida* and vaccine evaluation in bighorn sheep is justified because of the severe manifestations of pasteurellosis in bighorn sheep

populations and management goals. Use of a safe and effective vaccine against pathogenic strains of *Pasteurella* in bighorn sheep would be a valuable management technique, especially when bighorn sheep are captured or transplanted to new areas.

LITERATURE CITED

Biberstein, E. L. 1978. Biotyping and serotyping *Pasteurella haemolytica*. Pages 253-269 in T. Bergen and J.R. Norris, eds. Methods in microbiology. Academic Press, New York, NY.

Carter, G. R. 1984. Genus *Pasteurella*. Pages 552-558 in N. R. Krieg, ed. Bergey's manual of systemic bacteriology, Vol. 1. Williams and Wilkins. Baltimore, MD.

Cassirer, E. F., L. E. Oldenburg, V. L. Coggins, P. Fowler, K. Rudolf, D. Hunter, and W. J. Foreyt. 1996. Overview and preliminary analysis of a bighorn sheep dieoff, Hells Canyon 1995-1996. Bienn. Symp. North. Wild Sheep and Goat Counc. 10:78-86.

Foreyt, W. J. 1989. Fatal *Pasteurella haemolytica* pneumonia in bighorn sheep after direct contact with clinically normal domestic sheep. Am. J. Vet Res. 50:341-344.

____. 1990. Pneumonia in bighorn sheep: Effects of *Pasteurella haemolytica* from domestic sheep and effects on survival and long-term survival. Bienn. Symp. North. Wild Sheep and Goat Counc. 7:92-101.

____. 1992. Failure of an experimental *Pasteurella haemolytica* vaccine to prevent respiratory disease and death in bighorn sheep after exposure to domestic sheep. Bienn. Symp. North. Wild Sheep and Goat Counc. 8:155-163.

____. 1996. A 20-year health evaluation study of a healthy bighorn sheep population in northeastern Washington. Bienn. Symp. North. Wild Sheep and Goat Counc. 10:66-71.

____. 1997. Veterinary parasitology reference manual (4th ed.). Washington State University, Pullman, Washington, 190 pp.

____ , K. Snipes, and R. Kasten. 1994. Fatal pneumonia following inoculation of healthy bighorn sheep with *Pasteurella haemolytica* from healthy domestic sheep. J. Wildl. Dis. 30: 137-145.

____. And R. M. Silflow. 1996. Attempted protection of bighorn sheep (*Ovis canadensis*) from pneumonia using a nonlethal cytotoxic strain of *Pasteurella haemolytica*, biotype A, serotype 11. J. Wildl. Dis. 32:315-321.

Frank, G. H., and R. R. Wessman. 1978. Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. J. Clin. Microbiol. 7:142-145.

Kraabel, B. J, M. W. Miller, J. A. Conlon, and H. J. McNeil. 1998. Evaluation of a multivalent *Pasteurella haemolytica* vaccine in bighorn sheep: Protection from experimental challenge. J. Wildl. Dis. 34:325-333.

Martin, K. D., T. Schommer, and V. L. Coggins. 1996. Literature review regarding the compatibility between bighorn and domestic sheep. Bienn. Symp. North. Wild Sheep and Goat Counc. 10:72-77.

Miller, M. W., J. A. Conlon, H. J. McNeil, J. M. Bulgin, and C. S. Ward. 1997. Evaluation of a multivalent *Pasteurella haemolytica* vaccine in bighorn sheep: Safety and serologic responses. Wildl. Dis. 33:738-748.

Onderka, D. K., S. A. Rawlik, AND W. D. Wishart. 1988. Susceptibility of Rocky Mountain bighorn sheep and domestic sheep to pneumonia induced by bighorn and domestic livestock strains of *Pasteurella haemolytica*. Can. J. Vet. Res. 52:439-444.

Rimler, R. B., And K. A. Brogden. 1986. *Pasteurella multocida* isolated from rabbits and swine. Am. J. Vet. Res. 47:730-737.

Snipes, K. P., R. W. Kasten, M. A. Wild, M. W. Miller, D. A. Jessup, R. M. Silflow, W. J. Foreyt, and T. E. Miller. 1992. Using ribosomal RNA gene restriction patterns in distinguishing isolates of *Pasteurella haemolytica* from bighorn sheep (*Ovis canadensis*). J. Wildl. Dis. 28:347-354.

Wild, M. A., And M. W. Miller. 1991. Detecting nonhaemolytic *Pasteurella haemolytica* infections in healthy Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): Influences of sample site and handling. J. Wildl. Dis. 27:53-60.