

EVALUATION OF EWE VACCINATION AS A TOOL FOR INCREASING BIGHORN LAMB SURVIVAL FOLLOWING PASTEURELLOSIS EPIZOOTICS

Authors: Cassirer, E. Frances, Rudolph, Karen M., Fowler, Pat, Coggins, Victor L., Hunter, David L., et al.

Source: Journal of Wildlife Diseases, 37(1) : 49-57

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-37.1.49>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

EVALUATION OF EWE VACCINATION AS A TOOL FOR INCREASING BIGHORN LAMB SURVIVAL FOLLOWING PASTEURELLOSIS EPIZOOTICS

E. Frances Cassirer,^{1,7} Karen M. Rudolph,² Pat Fowler,³ Victor L. Coggins,⁴ David L. Hunter,^{2,6} and Michael W. Miller⁵

¹ Idaho Department of Fish and Game, 1540 Warner Avenue, Lewiston, Idaho 83501, USA

² Idaho Department of Fish and Game, Wildlife Health Lab, 16569 S. 10th Ave., Caldwell, Idaho 83605, USA

³ Washington Department of Fish and Wildlife, 8702 N. Division Street, Spokane, Washington 99218, USA

⁴ Oregon Department of Fish and Wildlife, 65495 Alder Slope Road, Enterprise, Oregon 97828, USA

⁵ Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA

⁶ Current address: Turner Enterprises, P.O. Box 190, Gallatin Gateway, Montana 59730, USA

⁷ Corresponding author (e-mail:fcassire@idfg.state.id.us)

ABSTRACT: We conducted field and laboratory experiments to evaluate whether treating pregnant bighorn ewes with a combination of an experimental *Pasteurella trehalosi* and *Mannheimia haemolytica* (formerly *P. haemolytica*) vaccine and a commercially-available bovine *P. multocida* and *M. haemolytica* vaccine would increase lamb survival following a pneumonia epidemic. Three free-ranging bighorn herds affected by pasteurellosis outbreaks between November 1995 and June 1996 were included in the field experiment. Post-epidemic lamb survival was low in all three herds in 1996, with November lamb:ewe ratios of $\leq 8:100$. In March 1997, thirty-six ewes (12/herd) were captured and radiocollared. Half of the ewes captured in each herd were randomly selected to receive both vaccines; the other half were injected with 0.9% saline solution as controls. Lambs born to radiocollared ewes were observed two or more times per week and were considered to have survived if they were alive in October 1997, about 6 mo after birth. Lamb survival differed among herds (range 22% to 100%), and survival of lambs born to vaccinated ewes was lower ($P = 0.08$) than survival of lambs born to unvaccinated ewes. Bronchopneumonia (pasteurellosis) was the dominant cause of mortality among lambs examined. We concurrently evaluated vaccine effects on survival of lambs born to seven captive ewes removed from the wild during the 1995–96 epidemic. Antibody titers were high in captive ewes prior to vaccination, and vaccines failed to enhance antibody titers in treated captive ewes. None of the captive-born lambs survived. These data suggest that, using existing technology, vaccinating bighorn ewes following pneumonia epidemics has little chance of increasing neonatal survival and population recovery.

Key words: Bighorn sheep, lamb mortality, *Ovis canadensis*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Pasteurella trehalosi*, pasteurellosis, pneumonia, vaccine.

INTRODUCTION

Periodic pneumonia-related epidemics are common in bighorn sheep (*Ovis canadensis*), and have limited successful restoration and management of this species. Although a variety of agents may be associated with these epidemics, bacteria in the genus *Pasteurella* (some species now included in the genus *Mannheimia*) appear to be the most common (Miller, 2000). In addition to acute mortality in affected bighorn populations, pasteurellosis (here used to describe diseases caused by both *Pasteurella* spp. and *Mannheimia* spp.) epidemics are typically followed by periods of poor neonatal survival that may last for several years (Onderka and Wis-

hart, 1984; Schwantje, 1986; Coggins, 1988; Festa-Bianchet, 1988). Typically, surviving bighorn ewes conceive and give birth, but lambs eventually succumb to pasteurellosis, presumably caused by bacteria transmitted from their dams (Foreyt, 1990; Miller, 2000). This extended period of low recruitment associated with pasteurellosis epidemics can have significant long-term impacts on bighorn sheep populations.

Numerous investigations into vaccination as a tool for preventing adult and lamb pasteurellosis have been conducted in domestic and wild sheep (Gilmour and Gilmour, 1989; Miller, 2000). Some vaccines simply failed to protect bighorn

sheep from pasteurellosis (Foreyt, 1992; Foreyt and Silflow, 1996), and others proved to be pathogenic in bighorns (Onderka et al., 1988). In contrast, more recent experimental vaccines containing leukotoxin and soluble cell surface antigens from *M. haemolytica* (formerly *P. haemolytica*) and *P. trehalosi* have offered significant protection against experimental challenge (Sutherland et al., 1989; Kraabel et al. 1998; Mosier et al., 1998). Miller et al. (1997) reported that vaccinating captive bighorn ewes increased leukotoxin neutralizing antibody titers for 12 to 16 wk and that vaccinating ewes 7 to 14 wk prior to parturition also appeared to elevate leukotoxin neutralizing antibody titers in colostrum. It follows that enhancing passive antibody transfer from vaccinated ewes to lambs through colostrum could potentially increase lamb survival by increasing immunity to *Pasteurella* spp. and *Mannheimia* spp. following pneumonia epidemics. Here, we describe field and laboratory experiments conducted to evaluate whether vaccinating free-ranging, pregnant bighorn ewes could increase lamb survival after a pneumonia epidemic.

MATERIALS AND METHODS

Efficacy of vaccination was evaluated concurrently in free-ranging and captive Rocky Mountain bighorn (*O. c. canadensis*) ewes that survived a pneumonia outbreak in the Hells Canyon area of Idaho, Oregon, and Washington, USA (Cassirer et al., 1996). Over 300 bighorn sheep died in this outbreak between November 1995 and June 1996. All-age mortality of free-ranging bighorns in three affected herds was estimated at 75% (165 sheep known dead) at Black Butte, Washington, USA (46°05'N, 116°55'W), 50% (60 sheep known dead) at Wenaha (Oregon, USA; 45°57'N, 117°30'W), and 5% (3 sheep known dead) at Redbird, Idaho, USA; 46°16'N, 116°57'W) (Cassirer et al., 1996). Nearly all lambs born into these herds during May to July 1996 died, and November 1996 lamb:ewe ratios were 8:100 (2 lambs/25 ewes) in the Black Butte herd (BB), 7:100 (2 lambs/28 ewes) in the Redbird herd (RB), and 3:100 (1 lamb/33 ewes) in the Wenaha herd (WE).

Necropsies conducted during the epidemic diagnosed *Pasteurella* spp.- or *Mannheimia*

spp.-associated bronchopneumonia as the cause of death in all cases examined (Cassirer et al., 1996). *Pasteurella multocida* was isolated from the lungs of 2 and *P. trehalosi* was isolated from the lungs of 4 of 11 pneumonic free-ranging sheep collected during the epidemic (Rudolph et al., 1999). Sixty-four of 72 bighorns captured in December 1995 and removed to a holding facility died in captivity with fibrinopurulent bronchopneumonia despite treatment that included oxytetracycline, penicillin, and ivermectin (D. Hunter, unpubl. data). *Pasteurella multocida* was isolated from the lungs of 42 and *P. trehalosi* was isolated from the lungs of 8 of 58 captive bighorns necropsied (Rudolph et al., 1999).

In our experiments, we used two vaccines in an attempt to provide broad protective immunity against bacteria that had been isolated from bighorns during the epidemic. For the field experiment, we used a split-plot design wherein individual bighorn ewes were randomly assigned to both treatment and control groups in each of three separate herds (BB, RB, and WE). Thirty-six adult ewes (12/herd) were captured by netgunning from a helicopter on 23 and 24 March 1997. Six ewes in RB and WE, and seven ewes in BB received experimental *P. trehalosi* and *M. haemolytica* vaccine (Miller et al., 1997; lot number 940902, no expiration date provided by manufacturer) injected into the right hind leg and commercially-available bovine *M. haemolytica/P. multocida* combination vaccine (PRESPONSE⁷ H-M, Fort Dodge Laboratories, Inc.; lot number 376205A, expiration date 08/15/97) injected into the left hind leg; a full recommended dose (2 ml, delivered intramuscularly) of each vaccine was given to each vaccinated bighorn. The other six ewes in RB and WE and five ewes in BB received 2 ml intramuscular injections of 0.9% saline solution in each hind leg as controls. At capture, blood and fecal samples and oropharyngeal swabs were collected, general body condition was evaluated based on visual observation, external parasites were sampled with ear swabs and by visual observation and manual collection, and age was estimated based on tooth wear and replacement. Each ewe also was ear-tagged and equipped with a unique, mortality-sensing radiocollar.

Free-ranging bighorn ewes and their lambs were observed two or more times per wk through binoculars and 40× or 60× spotting scopes between April and October 1997. We estimated lamb production and survival by observing whether or not the ewe was lactating, and whether or not the ewe was seen with a lamb at heel (Miller et al., 2000). If a ewe was seen alone or not observed to nurse a lamb

over several observation periods, it was assumed the lamb had died. In cases where the lamb was not recovered, we estimated mortality date as the midpoint between when the lamb was last seen and when the ewe was first seen without the lamb. A lamb was considered to have survived if it was alive at the end of October.

Lamb health was assessed by looking for signs of respiratory disease including coughing, nasal discharge, drooping ears, anorexia, lethargy, and segregation from the herd. Dead lambs were located by observing ewe behavior (Akenson 1998) and scanning cliffs for carcasses. Necropsies were conducted at the Idaho Department of Fish and Game Wildlife Health Laboratory (Caldwell, Idaho, USA; IWHL), or at the Washington State University Animal Disease and Diagnostic Laboratory (Pullman, Washington, USA; WADDL). Histopathological examinations and ancillary diagnostic tests were conducted at WADDL and the University of Idaho Caine Veterinary Teaching and Research Center (Caldwell, Idaho, USA; CVTRC).

The field experiment was accompanied by a smaller study on seven surviving ewes captured at BB and transferred to captivity at IWHL during the 1995–96 pasteurellosis epidemic. Captive bighorns were held in 3 to 5 ha paddocks and fed rations of grass/alfalfa hay daily; fresh water and mineralized salt blocks were provided *ad libitum*. Two of these ewes lambbed in captivity in 1996, but their lambs succumbed to pneumonia at about 4 wk postpartum. On 24 March 1997, four captive ewes were vaccinated with the two vaccines as described above, and three others were injected with saline as controls. Blood samples were collected at inoculation, 2 wk post-inoculation, and 11 wk post-inoculation. Blood samples were also collected from four captive-born lambs at 1 wk of age. Necropsies were conducted at the IWHL and diagnostic tests were conducted at CVTRC.

Levels of serum antibodies against *M. haemolytica* A1, A2, and *P. trehalosi* T10 serotype-specific surface antigens were measured using a direct microagglutination assay (Reggiardo, 1981) as described by Miller et al. (1997). Antibodies to serotype A1 and A2 antigens generally suggest exposure to *M. haemolytica* biogroup 1, and antibodies to serotype T10 generally suggest exposure to *P. trehalosi* biogroups 2 and 4CD (Ward et al., 1997; Jaworski et al., 1998); in all cases, some cross-reaction with other intraspecific serotypes may occur. Levels of leukotoxin neutralizing (LN) antibodies in bighorn sera were measured using a modified *in vitro* leukotoxin neutralization assay (Greer

and Shewen, 1985; Shewen and Wilke, 1988; Miller et al., 1997). All serological tests for *Pasteurella* spp. and *Mannheimia* spp. antibody titers were conducted at the Department of Pathobiology, University of Guelph, Ontario, Canada; titers were reported as reciprocal log₂ of endpoint dilutions for agglutination assays and as reciprocal log₂ dilution that yielded ≥50% neutralization of toxicity for leukotoxin neutralization assays.

Oropharyngeal swabs were cultured and *Pasteurella* spp. and *Mannheimia* spp. isolates biotyped at CVTRC using standard techniques (Kilian and Fredericksen, 1981; Bisgaard and Mutters, 1986; Ward et al., 1986, 1999). Serologic tests were conducted for antibodies to bluetongue virus (BTV) (Agar Gel Immunodiffusion [AGID], VMRD, Inc. Pullman, Washington, USA), epizootic hemorrhagic disease virus (EHDV) (AGID: Veterinary Diagnostic Technology, Inc. Wheat Ridge, Colorado, USA), bovine respiratory syncytial virus (BRSV) (Serum neutralization [SN]: neg@1:4, National Veterinary Services Laboratories [NVSL], Ames, Iowa; Testing Protocol 1998), parainfluenza-3 virus (PI-3) (SN:neg@1:4, NVSL Testing Protocol 1998), infectious bovine rhinotracheitis virus (IBRV) (SN: neg@1:4, NVSL Testing Protocol BPPR02104.02, 1998), bovine viral diarrhea virus (BVDV) (SN: neg@1:4, NVSL Testing Protocol 1998), *Brucella ovis* (Elisa: Walker et al., 1985), serovars of *Leptospira interrogans* (Microagglutination: neg@1:50), and *Anaplasma* spp. (Complement fixation: neg@1:5) at the Idaho State Department of Agriculture Laboratory (Boise, Idaho, USA). Fecal samples from 18 free-ranging bighorns were screened for intestinal parasites using a sugar flotation technique (Foreyt, 1994); results were reported as ova or cysts per g fresh feces (opg). Abundance of lungworm larvae (*Protostrongylus stilesi* and *P. rushi*), reported as larvae per g dried feces (lpg), was estimated using a modified Baermann technique (Beane and Hobbs, 1983). Pregnancy-specific protein B (PSPB) was measured to determine pregnancy in all bighorn ewes (Sasser et al., 1986; Noyes et al., 1997).

We analyzed differences in lamb survival among herds with a Pearson chi-square statistic and vaccine effect on lamb survival using the Cochran-Mantel-Haenszel chi-square statistic (SAS Institute, Inc., 1995). Differences in pre-existing antibody titers to *Pasteurella* spp. and *Mannheimia* spp. at capture among free-ranging bighorn ewes were analyzed via two-way analysis of variance (ANOVA) with subsequent treatment assignment and herd as main effects; student's *t*-tests were used for pairwise comparisons. Student's *t*-tests also were used to

TABLE 1. *Pasteurella* spp. and *Mannheimia* spp. biogroup variants isolated from oropharyngeal swabs collected from 27 free-ranging ewes in Idaho, Oregon, and Washington, March 1997.

Successful ^a ewes			Unsuccessful ewes		
Biogroup	Hemolysis	n	Biogroup	Hemolysis	n
2	+	3	2	+	2
2 ^B	–	5	2	–	2
3 ^{AE}	–	2	2 ^B	–	3
9 ^{ABL}	–	1	2 ^{BCD}	–	1
10 ^B	–	3	2 ^{BD}	–	1
4 ^{BLS}	–	2	2 ^D	–	1
1	+	1	2 ^D	+	2
U ^{AEL}	+	1	2 ^G	–	1
U ^L	+	2	4 ^B	–	1
			4 ^{BS}	–	1
			U ^{BLX}	–	1
<i>P. multo-</i>		2	<i>P. multo-</i>		1
<i>cida</i> A			<i>cida</i> B		

^a Successful or unsuccessful at raising a lamb May to October 1997.

compare titers between ewes with surviving lambs (successful) and ewes with lambs that died (unsuccessful). Differences in antibody titers in captive ewes before and after vaccination were evaluated with a paired *t*-test statistic (SAS Institute, Inc., 1995). Differences in macroparasite abundance in successful and unsuccessful ewes were evaluated with a Wilcoxon rank-sum statistic (Conover, 1980). Based on *a priori* calculations of experimental power (1 – β), we used α = 0.1 in assessing significance in all foregoing analyses.

RESULTS

Seventeen biogroup variants of *P. trehalosi* and *Mannheimia* spp. (originally reported as *P. haemolytica*) were isolated from oropharyngeal samples of 27 of 36 free-ranging bighorns. Seven biogroups occurred in more than one ewe: T^{2B}, T² (hemolytic and nonhemolytic), T^{2D}, T^{4BLS}, 3^{10B}, 3^{3AE}, and A^{UL}. *Pasteurella multocida* serotypes A (2 ewes) and B (1 ewe) also were isolated. Although most isolates were nonhemolytic (Table 1), hemolytic strains of both *P. trehalosi* and *Mannheimia* spp. were identified. No *Pasteurella* spp. or *Mannheimia* spp. were isolated from nine free-ranging bighorns sampled. *Mannheimia haemoytica* (5 of 9 samples) and *Pasteurella* spp. and *Mannheimia* spp. bio-

TABLE 2. *Pasteurella* spp. and *Mannheimia* spp. biogroup variants isolated from oropharyngeal swabs collected from seven captive bighorns at the Idaho Wildlife Health Laboratory (Caldwell, Idaho), March 1997.

Biogroup	Hemolysis	Number
2	–	1
2 ^B	–	7
2 ^{CDES}	+	1
4 ^{ACDE}	–	1
9 ^B	–	1
U ^{ABELX}	+	2
U ^{AL}	–	1
<i>P. multocida</i>		1
<i>P. multocida</i> A		1

variants T^{4BLS}, 3^{10B}, and 3^{3AE} were only isolated from the Redbird herd where all ewes successfully recruited a lamb. *Pasteurella trehalosi* biovariant T^{2B} occurred in all herds and biovariant T² occurred only in BB and WE. No discernable differences in the occurrence of *Pasteurella* spp. or *Mannheimia* spp. strains were observed between ewes that were ultimately successful or unsuccessful in recruiting lambs (Table 1).

Seven biogroup variants of *Mannheimia* spp., *P. trehalosi*, and *P. multocida* were isolated from seven captive ewes (Table 2).

In comparison to data reported elsewhere (Miller et al, 1997; Kraabel et al., 1998), prevaccination agglutinating titers to *M. haemolytica* serotype A2 and *P. trehalosi* serotype T10 were relatively high, as were leukotoxin neutralizing titers. Prevaccination agglutinating titers to serotype T10 and leukotoxin neutralizing titers differed among free-ranging herds (Table 3), but neither mean agglutinating antibody titers nor leukotoxin neutralizing titers differed (*P* > 0.50) between ewes that were subsequently assigned to treatment and control groups. Prevaccination antibody titers among captive ewes were similar to those in the free-ranging herds, except that titers to serotype A2 were lower in captive ewes (*P* < 0.01). Prevaccination agglutinating antibody titers to *P. trehalosi* serotype T10 and leukotoxin neutralizing

TABLE 3. Average agglutinating and leukotoxin neutralizing antibody titers to *Pasteurella* spp. and *Mannheimia* spp. prior to vaccination in three free-ranging and one captive bighorn sheep herds.

	Bighorn herd			<i>P</i> ^a	Captive
	Black Butte	Redbird	Wenaha		
LN ^b	8.00 (0.47)A ^d	7.46 (0.47)A	10.04 (0.47)B	0.002	7.50 (0.66)
Serotype A1 ^c	nd ^e	nd	nd		4.17 (0.61)
Serotype A2 ^c	7.91 (0.18)A	8.25 (0.17)A	7.92 (0.17)A	0.180	5.83 (0.24)
Serotype T10 ^c	9.36 (0.32)A	8.67 (0.30)A	10.67 (0.30)B	0.001	9.50 (0.42)

^a *P* value for ANOVA comparing herd effects on mean antibody titers among the three free-ranging herds.

^b Leukotoxin neutralizing antibody titers (1/log₂).

^c Agglutinating antibody titers (1/log₂).

^d Means (SE) followed by different letters are significantly different.

^e Not done.

titers were lower ($P < 0.05$) in free-ranging ewes that succeeded in recruiting a lamb than in unsuccessful ewes (Table 4). Vaccination of captive ewes did not significantly increase leukotoxin neutralizing or agglutinating titers at 2 or 11 wk after vaccination ($P > 0.18$) (Fig. 1). Average antibody titers of captive lambs at one week of age (A1 = 5, A2 = 6.75, T10 = 4, LN = 4.63) were 46 to 88% lower than those observed in ewes prior to vaccination.

All free-ranging ewes were negative for antibodies to BRSV, BTV, EHDV, and IBRV. One successful ewe had an antibody titer to BVDV (1:64), four ewes that reared lambs had *Leptospira interrogans* serovar *autumnalis* antibody titers (1:50), and three WE ewes that failed to raise lambs tested positive for antibodies against *Anaplasma* spp. (1:10). Ten free-ranging ewes had significant antibody titers to PI-3 (range 1:16 to 1:64), and seven of these were successful at raising lambs. Five of

seven captive ewes also had significant PI-3 antibody titers (range 1:32 to 1:64). All other serologic tests were negative in captive ewes.

Median abundance of *Nematodirus* spp. (3 opg), *Protostrongylus* spp. (13 lpg), and strongyles (0 opg) in free-ranging bighorns did not differ between successful and unsuccessful ewes. *Skrjabinema* spp. (median 15 opg) were found in samples from three bighorns. *Psoroptes* spp. mites were detected in four free-ranging ewes, and mild to moderate lesions but no mites were observed on another seven ewes.

Median lambing date was 20 May (range 30 April–26 July) for free-ranging bighorns and 28 May (range 21 May–30 June) for captive bighorns. We observed marked differences in lamb production and survival among the free-ranging herds ($P < 0.01$) (Table 5). Fourteen of 34 free-ranging pregnant ewes and all pregnant captive ewes either lost or failed to produce lambs. One pregnant captive ewe and one pregnant free-ranging ewe died before or during lambing and one captive ewe had twins. Five pregnant free-ranging ewes were never seen with a lamb and were assumed to have had stillborn lambs or lambs that died within 48 hr of birth. Mortality dates for lambs lost by the remaining eight free-ranging ewes were between 20 June and 12 October. Median age at mortality was 47 days for free-ranging lambs and 25 days for captive lambs.

TABLE 4. Comparison of average prevaccination *Pasteurella* spp. and *Mannheimia* spp. antibody titers in free-ranging bighorn ewes successful and unsuccessful at raising lambs during May to October 1997.

	Successful	Unsuccessful	<i>P</i>
LN ^a	7.89 (0.44) ^c	9.18 (0.46)	0.051
Serotype A2 ^b	8.16 (0.14)	7.88 (0.13)	0.145
Serotype T10 ^b	9.16 (0.29)	10.06 (0.32)	0.044

^a Leukotoxin neutralizing antibody titers (1/log₂).

^b Agglutinating antibody titers (1/log₂).

^c Standard error.

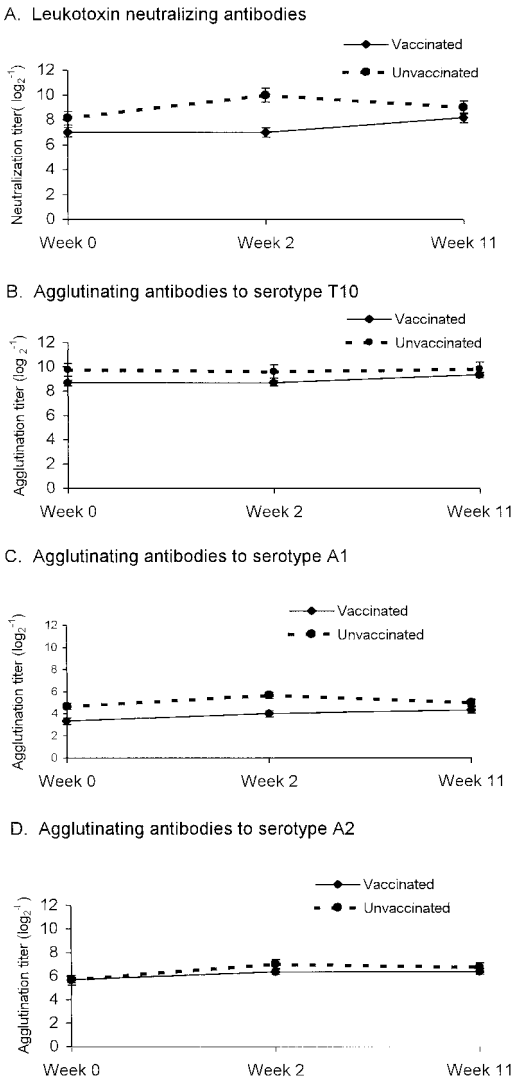


FIGURE 1. Antibody titers in response to vaccination in seven captive bighorns. Vertical lines are ± 1 standard error of mean observations.

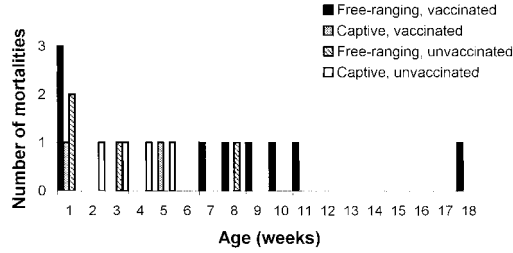


FIGURE 2. Age at death of free-ranging and captive bighorn lambs.

There was no difference in longevity of free-ranging lambs born to vaccinated or unvaccinated dams ($P = 0.13$), although median age at mortality in lambs from vaccinated dams ($n = 9$) was 57 days, whereas median age at death of lambs from unvaccinated dams ($n = 4$) was 10 days (Fig. 2). Overall, lamb survival was lower in vaccinated than unvaccinated free-ranging ewes ($P = 0.08$, Table 5).

Four of 13 dead free-ranging lambs (three from WE and one from BB) and the six dead captive lambs were collected and necropsied. Three of four free-ranging and five of the six captive lambs were diagnosed with acute bronchopneumonia. The remaining captive lamb died of starvation. The remaining free-ranging lamb was diagnosed with chronic bronchopneumonia complicated by traumatic peritonitis probably caused by aggression from an adult ewe; this ewe was observed butting the lamb repeatedly in the side prior to its death. *Pasteurella* spp. biotypes isolated from the lungs of five captive lambs and three free-ranging lambs included 2, 2^B, *P. multocida* A and *P. multocida* galli. No

TABLE 5. Ewe pregnancy and lamb survival rates in three free-ranging and one captive bighorn herd, March to October 1997.

Herd	Pregnant (%)	Number ewes observed with lambs (%)	Number surviving lambs (%) from all ewes	Number surviving lambs (%) from vaccinated ewes
Black Butte ($n = 12$)	12 (100)	8 (67)	5 (42)	2 (29)
Redbird ($n = 12$)	12 (100)	12 (100)	12 (100)	6 (100)
Wenaha ($n = 12$) ^a	10 (83)	7 (64)	2 (22)	0
Captive ($n = 7$) ^a	6 (86)	5 (83)	0	0

^a One pregnant Wenaha ewe and one pregnant captive ewe died before or during lambing.

Mannheimia spp. were cultured from the lungs of the dead lambs. No lungworm lesions were observed at necropsy and feces of one lamb tested were negative for all parasites. Signs of respiratory distress, especially coughing, lethargy, and isolation from the herd were recorded in most free-ranging lambs that died but could not be collected. In contrast, lambs at RB (where no lamb mortality occurred) were rarely observed to be lethargic, and were never observed coughing during 6 mo of field observation.

DISCUSSION

Two yr after an all-age pneumonia epizootic, lamb survival was much lower in two free-ranging bighorn herds (BB, WE) that experienced high mortality rates (50 to 75%) during the epidemic than in a herd (RB) where estimated mortality was low (5%) during the epidemic. Similarly, captive bighorn ewes that survived an epidemic where mortality rates were high (89%) were completely unsuccessful at raising lambs 2 yr later. Pneumonic pasteurellosis appeared to be the predominant cause of mortality of both free-ranging and captive bighorn lambs. Several lambs recovered had *P. multocida* or *P. trehalosi* biovariants in the lungs that were not found on oropharyngeal swabs collected from their respective dams prior to lambing. It is possible that these strains were present but not detected in the ewe; alternatively, they may have been transmitted to the dam after sampling but prior to parturition or transmitted from another ewe or lamb to these lambs and/or their dams after parturition. None of the *Pasteurella* spp. strains isolated from the lungs of dead lambs appeared to have been introduced: all had been recovered from one or more ewes in their respective populations prior to parturition. Moreover, these isolates represented biovariants commonly found in the tonsils and nasal passages of clinically healthy bighorn sheep elsewhere (Queen et al., 1994; Jaworski et al., 1998), and some ewes carrying these biovariants

successfully raised lambs in this experiment. However, no other likely initiating pathogens were found during necropsies or in the ewes sampled at capture.

Vaccinating pregnant ewes 5 to 12 wk prior to parturition was safe, but had no beneficial effect on lamb survival. In both free-ranging and captive ewes studied here, prevaccination leukotoxin neutralization titers were at or above postvaccination titers reported during previous vaccine studies on captive bighorns in Colorado, USA (Miller et al., 1997; Kraabel et al., 1998). The failure of vaccination to increase antibody titers in captive ewes may have been due to preexisting high antibody levels; similar unresponsiveness in individuals with high preexisting antibody titers has been reported in other *Pasteurella* spp. and *Mannheimia* spp. vaccine studies in bighorn and domestic sheep (Ward et al., 1999: Fig. 4C) and cattle (Hodgins and Shewen, 1994). If our observations are representative of antibody titers and likely responses in bighorn ewes following epidemics, then vaccination would be more effective in stimulating immunity in naive populations.

Antibody titers to *Pasteurella* and *Mannheimia* spp. in ewes, although high, apparently were not sufficient to enhance lamb survival. In fact, differences in agglutinating titers to serotype T10 and in leukotoxin neutralizing titers (Table 4) suggested that ewes with lower prevaccination antibody titers were actually more likely to be successful in recruiting lambs. This finding, although initially counterintuitive, is consistent with previous observations of interference between passive immunity and development of acquired immunity in a variety of animal species, including domestic sheep and cattle (Gilmour et al., 1980; Kiorpes et al., 1991; Hodgins and Shewen, 1994; Gershwin et al., 1995; Tizard, 1996). It follows that vaccinating ewes actually may have exacerbated such interference, thereby offering a plausible underlying mechanism for observed re-

duction in survival among lambs born to vaccinated ewes.

Free-ranging lambs appeared especially vulnerable to pasteurellosis from 6 to 11 wk of age, near the time that passively-acquired agglutinating and leukotoxin neutralizing antibody levels wane (Miller et al., 1997); however, one lamb succumbed to pasteurellosis in October, long after passive immunity would be expected to be protective. Thus, even if vaccination can initially enhance passive immunity to pasteurellosis, lambs still may eventually succumb in herds where virulent strains of *Pasteurella* spp. and/or *Mannheimia* spp. are circulating. Based on our findings it appears that, given existing technology, vaccinating bighorn ewes following pneumonia epidemics has little chance of increasing neonatal survival, and may be contraindicated as a management intervention.

ACKNOWLEDGMENTS

This study was supported by the Foundation for North American Wild Sheep, Idaho Department of Fish and Game, Oregon Department of Fish and Wildlife, Washington Department of Fish and Wildlife, and Colorado Division of Wildlife. We appreciate the assistance of H. Akenson, C. Kallstrom, D. Carroll, G. Majors, J. Mulholland, A. C. S. Ward, H. McNeil, J. Conlon, and numerous other individuals who helped with various aspects of designing and conducting this study.

LITERATURE CITED

- AKENSON, H. A. 1998. Predicting summer lamb mortality in free ranging bighorn sheep. Biennial Symposium of the Northern Wild Sheep and Goat Council 11: 70–76.
- BEANE, R. D., AND N. T. HOBBS. 1983. The Baermann technique for estimating *Protostrongylus* infection in bighorn sheep: Effect of laboratory procedures. Journal of Wildlife Diseases 19: 7–9.
- BISGAARD, M., AND R. MUTTERS. 1986. Re-investigations of selected bovine and ovine strains previously classified as *Pasteurella haemolytica* and description of some new taxa within the *Pasteurella haemolytica*-complex. Acta Pathologica, Microbiologica et Immunologica Scandinavica. Section B. Microbiology 94: 185–193.
- CASSIRER, E. F., L. E. OLDENBURG, V. L. COGGINS, P. FOWLER, K. M. RUDOLPH, D. L. HUNTER, AND W. J. FOREYT. 1996. Overview and preliminary analysis of Hells Canyon bighorn sheep die-off, 1995–96. Biennial Symposium of the Northern Wild Sheep and Goat Council 10: 78–86.
- COGGINS, V. L. 1988. The Lostine Rocky Mountain bighorn sheep die-off and domestic sheep. Biennial Symposium of the Northern Wild Sheep and Goat Council 6: 57–64.
- CONOVER, W. J. 1980. Practical nonparametric statistics, 2nd Edition. John Wiley & Sons, New York, New York, 493 pp.
- FESTA-BIANCHET, M. 1988. A pneumonia epizootic in bighorn sheep, with comments on preventive management. Biennial Symposium of the Northern Wild Sheep and Goat Council 6: 66–76.
- FOREYT, W. J. 1990. Pneumonia in bighorn sheep: Effects of *Pasteurella haemolytica* from domestic sheep and effects on survival and long-term reproduction. Biennial Symposium of the Northern Wild Sheep and Goat Council 7: 92–101.
- . 1994. Veterinary parasitology reference manual. Wash. State Univ., Pullman, Washington, 178 pp.
- GERSHWIN, L. J., S. KRAKOWA, AND R. G. OLSEN. 1995. Immunology and Immunopathology of Domestic Animals, 2nd Edition. Mosby, Saint Louis, Missouri, 195 pp.
- GILMOUR, N. J. L., AND J. S. GILMOUR. 1989. Pasteurellosis of sheep. In *Pasteurella* and Pasteurellosis, C. Adlam and J. M. Rutter (eds.). Academic Press Ltd., San Diego, California, pp. 223–262.
- , J. M. SHARP, W. DONACHIE, C. BURRELLS, AND J. FRASER. 1980. Serum antibodies of ewes and their lambs to *Pasteurella haemolytica*. The Veterinary Record 107: 505–507.
- GREER, C. N., AND P. E. SHEWEN. 1986. Automated colorimetric assay for the detection of *Pasteurella haemolytica* leukotoxin. Veterinary Microbiology 12: 33–42.
- HODGINS, D. C., AND P. E. SHEWEN. 1994. Passive immunity to *Pasteurella haemolytica* A 1 in dairy calves: Effects of periparturient vaccination of the dams. Canadian Journal of Veterinary Research 58: 31–35.
- JAWORSKI, M. D., D. L. HUNTER, AND A. C. S. WARD. 1998. Biovariants of isolates of *Pasteurella* from domestic and wild ruminants. Journal of Veterinary Diagnostic Investigation 10: 49–55.
- KILIAN, M., AND W. FREDERIKSEN. 1981. Identification tables for the *Haemophilus-Pasteurella-Actinobacillus* group. In *Haemophilus, Pasteurella, and Actinobacillus*. M. Kilian, W. Frederiksen, and E. L. Biberstein (eds.). Academic Press Ltd., San Francisco, California, pp. 281–287.
- KIROPES, A. L., M. T. COLLINS, T. P. GOERKE, D. L. TRAIL, A. W. CONFER, M. J. GENTRY, AND L. E. BAUMANN. 1991. Immune response of neonatal lambs to a modified live *Pasteurella haemolytica* vaccine. Small Ruminant Research 4: 73–87.

- 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. *Journal of Wildlife Diseases* 34: 325–333.
- MILLER, M. W. 2000. Pasteurellosis. In *Infectious diseases of wild mammals*, 3rd edition, E. S. Williams and I. K. Barker, (eds.). Iowa State University Press, Ames, Iowa, In press.
- , J. A. CONLON, H. J. MCNEIL, J. M. BULGIN, AND A. C. S. WARD. 1997. Evaluation of a multivalent *Pasteurella haemolytica* vaccine in bighorn sheep: Safety and serologic responses. *Journal of Wildlife Diseases* 33: 738–748.
- , J. E. VAYHINGER, D. C. BOWDEN, S. P. ROUSH, T. E. VERRY, A. N. TORRES, AND V. D. JURGENS. 2000. Drug treatment for lungworm in bighorn sheep: Reevaluation of a 20-year-old management prescription. *Journal of Wildlife Management* 64: 505–512.
- MOSIER, D. A., R. J. PANCIERA, D. P. ROGERS, G. A. ULICH, M. D. BUTINE, A. W. CONFER, AND R. J. BASARABA. 1998. Comparison of serologic and protective responses induced by two *Pasteurella* vaccines. *Canadian Journal of Veterinary Research* 62: 178–182.
- NOYES, J. H., R. G. SASSER, B. K. JOHNSON, L. D. BRYANT, AND B. ALEXANDER. 1997. Accuracy of pregnancy detection by serum protein (PSPB) in elk. *Wildlife Society Bulletin* 25: 695–698.
- ONDERKA, D. K., AND W. D. WISHART. 1984. A major bighorn sheep dieoff from pneumonia in southern Alberta. *Biennial Symposium of the Northern Wild Sheep and Goat Council* 4: 356–363.
- , S. A. RAWLUK, 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*. *Canadian Journal of Veterinary Research* 52: 439–444.
- QUEEN, C., A. C. S. WARD, AND D. L. HUNTER. 1994. Bacteria isolated from nasal and tonsillar samples of clinically healthy Rocky Mountain bighorn and domestic sheep. *Journal of Wildlife Diseases* 30: 1–7.
- REGGIARDO, C. 1981. Serological tests in bovine bacterial pneumonias. *Epidemiological application and potential as a research tool. Advances in Experimental Medicine and Biology* 137: 818–822.
- RUDOLPH, K. M., D. L. HUNTER, R. B. RIMLER, E. F. CASSIRER, W. J. FOREYT, W. J. DELONG, AND A. C. S. WARD. 1999. Micro-organisms associated with a bighorn sheep pneumonia epizootic in Hells Canyon, USA. Idaho Department of Fish and Game, Wildlife Health Laboratory, Caldwell, Idaho, 21 pp.
- SAS INSTITUTE, INC. 1995. Statistical analysis system user's guide, statistics. SAS Institute Incorporated, Cary, North Carolina, 1686 pp.
- SASSER, R. G., C. A. RUDER, K. A. IVANI, J. E. BUTLER, AND W. C. HAMILTON. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. *Biology of Reproduction* 35: 936–942.
- SCHWANTJE, H. M. 1986. A comparative study of bighorn sheep herds in southeastern British Columbia. *Biennial Symposium of the Northern Wild Sheep and Goat Council* 5: 231–252.
- SHEWEN, P. E., AND B. N. WILKIE. 1988. Vaccination of calves with leukotoxic culture supernatant from *Pasteurella haemolytica*. *Canadian Journal of Veterinary Research* 52: 30–36.
- SUTHERLAND, A. D., W. DONACHIE, G. E. JONES, AND M. QUIRIE. 1989. A crude cytotoxin protects sheep against experimental *Pasteurella haemolytica* serotype A2 infection. *Veterinary Microbiology* 19: 175–181.
- TIZARD, I. R. 1996. *Veterinary Immunology*, 5th Edition. W. B. Saunders, Philadelphia, Pennsylvania, 531 pp.
- WALKER, R. L., B. R. LEAMASTER, J. N. STELLFLUG, AND E. L. BIBERSTEIN. 1985. Use of enzyme-linked immunosorbent assay for detection of antibodies to *Brucella ovis* in sheep: Field trial. *American Journal of Veterinary Research* 46: 1642–1646.
- WARD, A. C. S., D. L. HUNTER, M. D. JAWORSKI, P. J. BENOLKIN, M. P. DOBEL, J. B. JEFFRIES, AND G. A. TANNER. 1997. *Pasteurella* spp. in sympatric bighorn and domestic sheep. *Journal of Wildlife Diseases* 33: 544–557.
- , ———, K. M. RUDOLPH, W. J. DELONG, J. M. BULGIN, L. M. COWAN, H. J. MCNEIL, AND M. W. MILLER. 1999. Immunologic responses of domestic and bighorn sheep to a multivalent *Pasteurella haemolytica* vaccine. *Journal of Wildlife Diseases* 35: 285–296.
- , L. R. STEVENS, B. J. WINSLOW, R. P. GOGOLEWSKI, D. C. SCHAEFER, S. K. WATSON, AND B. L. WILLIAMS. 1986. Isolation of *Haemophilus somnus*: A comparative study of selective media. *American Association of Veterinary Laboratory Diagnosticians* 29: 479–486.

Received for publication 6 July 1999.