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Authors: Miller, Michael W., Conlon, Jennifer A., McNeil, Heather J., Bulgin, Jeanne M., and Ward, Alton C. S.

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EVALUATION OF A MULTIVALENT *PASTEURELLA HAEMOLYTICA* VACCINE IN BIGHORN SHEEP: SAFETY AND SEROLOGIC RESPONSES

Michael W. Miller,[1] Jennifer A. Conlon,[2,5] Heather J. McNeil,[3,6] Jeanne M. Bulgin,[4] and Alton C. S. Ward[4]

[1] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA
[2] Fort Dodge Laboratories, 800 Fifth Street Northwest, Fort Dodge, Iowa 50501, USA
[3] Ayerst Veterinary Laboratories, 131 Malcolm Road, Guelph, Ontario N1K 1A8, Canada
[4] University of Idaho, Caine Veterinary Teaching and Research Center, 1020 East Homedale Road, Caldwell, Idaho 83605-8098, USA
[5] Present address: Rhone Merieux, Incorporated, 115 Transtech Drive, Athens, Georgia 30601, USA
[6] Present address: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: We examined effects of a multivalent *Pasteurella haemolytica* vaccine (serotypes A1, A2, T10) on humoral immune responses and *P. haemolytica* isolation rates in bighorn sheep (*Ovis canadensis*). Thirty captive bighorns, divided into groups of three on the basis of age, sex, and previous history of pneumonic pasteurellosis, received 0, 1, or 2 vaccine doses. Mild, transient lameness in most bighorns 1 day after initial vaccination was the only adverse effect observed. Oropharyngeal (≥75%) and nasal (≤50%) isolation rates for *P. haemolytica* did not differ among treatment groups. Ten of 36 distinguishable biogroup variants accounted for about 87% of the 464 *P. haemolytica* isolates from bighorns, but prevalences of specific biogroups were not affected by vaccination. Bighorns receiving 1 or 2 vaccine doses showed marked elevations in leukotoxin neutralizing antibody titers beginning 1 wk after vaccination. Agglutinating antibody titers to serotype A1 and A2 surface antigens were also elevated in vaccinated bighorns within 2 wk after vaccination; agglutinating antibody titers to serotype T10 surface antigens were relatively high in all three groups but appeared unaffected by vaccination. Vaccination 7 to 14 wk prior to parturition elevated leukotoxin neutralizing antibody titers in colostrum, but neither leukotoxin neutralizing nor serotype A1 surface antigen agglutinating antibody titers differed through 16 wk of age among lambs born to dams from different vaccine dose groups. Our data demonstrate that this multivalent *P. haemolytica* vaccine is safe and stimulates marked antibody responses in bighorn sheep. Further evaluation of this vaccine as a tool in preventing and managing pasteurellosis in bighorn sheep appears warranted.

Key words: Bighorn sheep, experimental study, *Ovis canadensis*, *Pasteurella haemolytica*, pasteurellosis, pneumonia, serology, vaccine.

INTRODUCTION

Successful bighorn sheep (*Ovis canadensis*) management appears to be dependent on mitigating or eliminating pneumonia epizootics in otherwise thriving herds. Periodic pneumonia epizootics cause extensive mortality in bighorn sheep populations throughout North America (Rush, 1927; Potts, 1937; Marsh, 1938; Buechner, 1960; Onderka and Wishart, 1984; Spraker et al., 1984; Festa-Bianchet, 1988). Although viral, bacterial, and parasitic agents have all been incriminated in these epizootics, *Pasteurella* spp. are perhaps the most common pathogens associated with bronchopneumonia in bighorns. Two species, *P. haemolytica* and *P. multocida*, and several biotypes and/or serotypes within those species, have been isolated from bighorns during epizootics (Post, 1962; Onderka and Wishart, 1984; Spraker et al., 1984; Miller et al., 1991a, b).

Despite extensive diagnostic and experimental investigation, the epizootiology of pasteurellosis in wild bighorn populations is poorly understood. Hypotheses regarding the ecology and epizootiology of pneumonia epizootics in bighorn sheep have not been rigorously tested, and the relative importance of endogenous and introduced *Pasteurella* spp. strains as factors limiting bighorn abundance remains particularly unclear (Miller et al., 1991a, b; Wild and

TABLE 1. Block and random treatment assignments for captive bighorn sheep used in evaluation of a multivalent *Pasteurella haemolytica* vaccine.

Strata			Treatment group[a]		
Age	Sex	Health history[b]	0	1	2
<1 yr	Either	H	L894[c]	C994	A94
		P	C294	Q94	L794
>1 yr	Male	H	M87	A93	M92
		P	M86	E992	L92
>1 yr	Female	H	L289	E83	T88
	(nonpregnant)	P	M91	A82	E88
>1 yr	Female	H	M88	C89	E89
	(pregnant)		M93	L93	C92
		P	Q92	A85	E392
			L87	L88	Q86

[a] 0 = (control, no vaccination); 1 = (1 vaccine dose); 2 = (2 vaccine doses 14 days apart).
[b] H = (no history of pneumonic pasteurellosis); P = (history of pneumonic pasteurellosis).
[c] Individual bighorns as identified by alphanumeric ear tags.

Miller, 1991; Hobbs and Miller, 1992). In the absence of knowledge about the epizootiology of pasteurellosis, few strategies for effectively managing pneumonia in bighorn populations have emerged. Wildlife managers cannot predict epizootics in bighorns, and proposed strategies for preventing such epizootics remain largely untested. Consequently, long-term attempts to manage bighorns often fail.

Difficulties in understanding and managing pasteurellosis also plague the domestic sheep industry world-wide. Reported differences in species susceptibility and strain-specific pathogenesis notwithstanding (Onderka and Wishart, 1988; Onderka et al., 1988; Silflow et al., 1989, 1993), the epizootiology of pasteurellosis in domestic sheep (reviewed by Gilmour and Gilmour, 1989) is strikingly similar to that observed in bighorn sheep (Foreyt, 1990; Miller et al., 1991a, b; Hobbs and Miller, 1992). Because of its wide distribution, sporadic nature and significant economic impacts, recent attempts to manage pasteurellosis in domestic sheep have focused on prevention through vaccination. The efficacies of vaccines developed for domestic sheep have varied widely (Gilmour and Gilmour, 1989; Donachie, 1995), and many either exacerbated or failed to prevent disease. However, experimental vaccines containing leukotoxin and soluble cell surface antigens from *P. haemolytica* offered ≥37% protection against experimental challenge (Sutherland et al., 1989; Alexander et al., 1995). Humoral immune responses stimulated by one of these vaccines (Sutherland et al., 1989) approximated those observed in lambs allowed to recover from experimental *P. haemolytica* infections (Donachie et al., 1986). Because captive bighorn sheep that survived pneumonic pasteurellosis have shown resistance during subsequent pneumonia epizootics (Miller et al., 1991b), it follows that vaccines stimulating antibody to *P. haemolytica* leukotoxin and soluble cell surface antigens might afford protection against naturally occurring pasteurellosis in bighorns as well. Here, we examined effects of an experimental, multivalent *P. haemolytica* vaccine (serotypes A1, A2, T10) on humoral immune responses and *P. haemolytica* isolation rates in captive bighorn sheep.

MATERIALS AND METHODS

We used 30 captive Rocky Mountain bighorn sheep (*O. canadensis canadensis*) (Table 1) in this experiment. All bighorns were housed at the Colorado Division of Wildlife's Foothills Wildlife Research Facility (Fort Collins, Colorado, USA; 40°35′N, 105°10′W) throughout the study. We subdivided bighorns into groups by

age and sex (<1 yr, >1 yr rams, >1 yr nonpregnant ewes, >1 yr pregnant ewes) (Table 1), and individuals within these subgroups resided together in 3 to 7 ha pastures throughout the study. In addition to natural forage, grass/alfalfa hay mix and a pelleted high-energy supplement were provided throughout the study as prescribed under established feeding protocols for bighorn sheep in respective age/sex classes (Miller, 1990); fresh water and mineralized salt blocks were also provided *ad libitum*.

The general health of all bighorns was evaluated prior to and immediately after vaccination, and daily thereafter. We recorded health observations throughout the experiment, giving particular attention to detecting signs of respiratory disease (depression, segregation, anorexia, nasal discharge, coughing, labored breathing). Injection sites were examined weekly for 4 wk after vaccine administration to assess local reactions. All bighorns were weighed in conjunction with sampling. Health problems were evaluated and treated by attending veterinarians as necessary. Bighorns that died during our experiment were submitted to the Colorado State University Diagnostic Laboratory (Fort Collins, Colorado, USA) or Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA), where carcasses were necropsied and subjected to histopathologic examination and ancillary diagnostic tests to determine the cause of death.

The experimental *P. haemolytica* vaccine (Langford Laboratories, Inc., Guelph, Ontario, Canada; lot 940902) used here was a bacterial cell-free extract of culture supernatants from three *P. haemolytica* serotypes (A1, A2, T10) that contained leukotoxin, serotype-specific surface antigens, and an adjuvant (*MUNOKYN-IN*™, Langford Laboratories, Inc., Kansas City, Missouri, USA). Methods for preparation and serotype A1 components of this vaccine were the same as those used in a commercially-available bovine vaccine (PRESPONSE®, Langford Laboratories, Inc.).

We measured the effects of vaccine administration on humoral immune responses and *P. haemolytica* isolation rates in captive bighorn sheep, and on antibody levels and isolation rates in lambs born to pregnant ewes included in our study. Resistance to experimental challenge with pathogenic *P. haemolytica* was not tested in this study, but we did not attempt to prevent the natural occurrence of pneumonic pasteurellosis in study bighorns.

Our study was designed as a randomized complete block experiment with a repeated measures structure. In order to distribute treatments equally across the study population, subject animals were stratified by age (<1 yr, >1 yr), sex (>1 yr rams, >1 yr nonpregnant ewes, >1 yr pregnant ewes), and previous history of pneumonic pasteurellosis (health history) (Table 1). Within strata, individual sheep were assigned to blocks (n = 3 animals/block) (Table 1). One bighorn within each block was then randomly assigned to each of 3 treatment groups: 0 (control, no vaccination), 1 (1 vaccine dose), or 2 (2 vaccine doses 14 days apart) (Table 1).

On 20 February 1995 (=wk 0), we aseptically injected 2 ml of experimental vaccine intramuscularly (IM) into bighorns in treatment groups 1 and 2; controls (group 0) were injected IM with 2 ml 0.9% saline. Fourteen days later, bighorns in treatment group 2 were injected IM with a second 2 ml vaccine dose; bighorns in treatment groups 0 and 1 were injected IM with 2 ml 0.9% saline. All bighorns received vaccine or saline in the right hind leg on day 0 and in the left hind leg on day 14.

We collected about 10 to 12 ml blood for antibody measurements from each bighorn on wk 0 (prior to vaccination), and on weeks 1, 2, 3, 4, 6, 8, 12, 16, 25 and 29 postvaccination; we also collected oropharyngeal and nasal swabs (Baxter Healthcare Corporation, McGaw Park, Illinois, USA) from each bighorn on wk 0, 2, 4, 8, 12 and 29. Blood and oropharyngeal swabs were collected from ewes and their lambs within 12 hr postpartum, and about 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 wk postpartum; we also collected colostrum from ewes within 12 hr postpartum. Blood samples were held for 1 to 4 hr at about 22 C, centrifuged, and serum collected. Serum and colostrum were stored at −20 C until analyzed at Ayerst Veterinary Laboratories (Guelph, Ontario, Canada). Swabs were placed in transport tubes containing modified Cary and Blair medium (Port-A-Cul®, Becton Dickinson Microbiology Systems, Becton Dickinson and Company, Cockeysville, Maryland, USA) and shipped overnight on ice packs to the University of Idaho's Caine Veterinary Teaching and Research Center (CVTRC; Caldwell, Idaho, USA) for culture and analysis.

Levels of leukotoxin neutralizing antibodies in bighorn sera and colostrum were measured using a modified *in vitro* leukotoxin neutralization assay (Greer and Shewen, 1986; Shewen and Wilke, 1988). Serial two-fold dilutions of test sera or colostrum were preincubated with leukotoxic *P. haemolytica* culture supernatant for 30 min at 22 C; supernatant concentration was adjusted beforehand to produce a standardized titer with positive control bovine serum. We then transferred 50 µl of each serum (or colostrum)/toxin mixture to microtiter plate wells containing bovine leukemia-derived B cell line (BL-3) cells and 50 µl Roswell Park Memorial Institute (RPMI) medium (Life

Technologies/Gibco BRL, Toronto, Ontario, Canada). Plates were incubated for 1 hr at 37 C, and cell viability was measured by uptake of neutral red dye as previously described (Greer and Shewen, 1986). All sera were assayed in duplicate, along with positive and negative control bovine sera. We expressed neutralization titers as the highest reciprocal $\log_2$ dilution that yielded ≥50% neutralization of toxicity. Levels of serum and colostral antibodies against serotype-specific surface antigens were measured using a direct microagglutination assay (Shewen and Wilkie, 1982) that incorporated washed formalinized *P. haemolytica* serotype A1, A2, or T10 cells as antigen. We expressed agglutination titers as the reciprocal $\log_2$ of endpoint dilutions.

At CVTRC, oropharyngeal and nasal swabs were plated on both Columbia blood agar (Difco Laboratories, Detroit, Michigan, USA) with 5% citrated ovine blood (CBA) and a selective medium containing Columbia blood agar, 7% bovine blood, and antibiotics selective for Pasteurellaceae (Ward et al., 1986). Suspected *P. haemolytica* colonies were selected after 48 hr incubation at 37 C in 5% CO_2 and further propagation was carried out on CBA. Plates were examined for hemolysis. Identities and biochemical profiles of colonies yielding gram negative rods or coccobacilli that fermented triple sugar iron, glucose, and sucrose were further evaluated to determine species and biogroup identifications (Kilian and Fredericksen, 1981; Bisgaard and Mutters, 1986). The biogrouping schema of Bisgaard and Mutters (1986) was modified by CVTRC to allow use of negative test results to separate isolates into additional biogroups. Select isolates identified as *P. haemolytica* were further characterized by serotype using rapid plate agglutination (Frank and Wessman, 1978). We calculated rates for isolating *P. haemolytica,* both in general and for select biogroups, from nasal and oropharyngeal sites.

Although resistance to experimental challenge with pathogenic *P. haemolytica* was not tested in this study, we observed bighorns daily for signs of respiratory disease (nasal discharge, depression, segregation, anorexia, coughing, dyspnea). We defined clinical pneumonia as a combination of signs including mucopurulent nasal discharge, depression (with or without segregation from penmates), anorexia or failure to gain weight, and coughing or dyspnea, accompanied by estimated resting body temperature ≥39.5 C. The probable etiology of all pneumonia cases was determined by ancillary diagnostic tests.

We compared levels of neutralizing antibody titers to *P. haemolytica* leukotoxin and levels of antibody to *P. haemolytica* surface antigens in serum and colostrum, rates of *Pasteurella* spp. isolation from oropharyngeal and nasal sites, phenotypic traits of *P. haemolytica* isolates, and rates of naturally-occurring pneumonic pasteurellosis among treatment groups. We analyzed serologic data using least squares analysis of variance for general linear models (SAS Institute, Inc., 1989). Responses to treatments were analyzed with analysis of variance for a randomized complete block design with a repeated measures structure. We used vaccine doses (0, 1, 2), age/sex and health history as main effects. Factors in the analysis were vaccine treatment, age/sex, health history, and interaction of vaccination and health history. Time was treated as a within subject effect using a multivariate approach to repeated measures (Morrison, 1976). For postpartum serum and colostrum titer data, we also used least squares analysis of variance for a randomized complete block design with vaccine dose as the sole main effect. We calculated prevalence rates for *P. haemolytica* isolation from oropharyngeal and nasal sites and compared these among treatments using Fisher's exact probability tests (Mielke and Berry, 1992). We also compared prevalences of phenotypically distinct strains of *P. haemolytica* among treatments for the ten most common biogroups isolated using Fisher's exact probability tests. For all comparisons, we used $\alpha = 0.05$ in assessing statistical significance.

RESULTS

Mild, transient lameness in most bighorns 1 day after initial vaccination was the only adverse effect observed. We observed no clinical signs of pneumonic pasteurellosis in any of the study bighorns. One ewe (L93) died, but her death appeared unrelated to vaccine treatment: she succumbed to a pyogranulomatous brain abscess that was probably caused by a migrating grass awn about 4.5 mo after vaccination; *Actinomyces pyogenes* was isolated from this lesion. We observed no abortifacient effects associated with vaccinating pregnant ewes ($n = 13$); no definite etiology was ascribed to one abortion that occurred in early April, but the affected ewe (A82) had a preexisting (≥2 yr) history of late-term abortions. Of 12 lambs delivered between 10 April and 23 May, eight survived to weaning. All lamb mortality occurred ≤5 days after birth. We attributed two of the

four perinatal losses to hypothermia (possibly complicated by prematurity), one to starvation, and one to stillbirth possibly caused by prematurity and/or inadequate maternal care; evidence of infectious disease was not observed in any of these lambs. None of the losses appeared to be related to vaccine treatment: two of the ewes that lost lambs had been vaccinated and two were controls. We observed no signs of pneumonic pasteurellosis in any of the eight surviving lambs.

We recovered *P. haemolytica* from all captive bighorns used in this study. Oropharyngeal (≥75%) and nasal (≤50%) isolation rates for *P. haemolytica* did not differ among treatment groups ($P \geq 0.43$). Serotyping results varied among and within biogroups, and most isolates reacted to either all or none of the antisera used for testing *P. haemolytica* biotype A or T isolates. Because serotype data appeared less consistent and informative than biogroup data, we used the latter in evaluating treatment effects on isolation rates. In all, we identified 36 distinguishable biogroup variants among 464 *P. haemolytica* isolates from adult bighorns. We observed differences in relative prevalence of specific *P. haemolytica* biogroups: ten biogroup variants accounted for about 87% of all isolates (Table 2). Prevalence within respective biogroups was not affected by vaccine treatment ($P \geq 0.185$), although some temporal trends in relative prevalence were evident (Table 2). Vaccination of dams also had no apparent effect on colonization of lambs: we initially isolated *P. haemolytica* from all neonatal lambs <1 to 16 days after birth and routinely thereafter.

Mean pretreatment titers for *P. haemolytica* leukotoxin neutralizing antibody were relatively low (≤0.7) across all three treatment groups (Fig. 1A), and remained low (≤2.1) throughout the 29 wk trial in unvaccinated bighorns. In contrast, vaccinated bighorns showed marked elevations ($P = 0.0001$) in leukotoxin neutralizing antibody titers beginning 1 wk after initial vaccination ($P = 0.0001$). Mean responses peaked at 2 wk for both vaccine groups, and titers remained elevated above control titers ≥8 but <12 wk in group 1 ($P \leq 0.0014$) and ≥16 but <25 wk in group 2 ($P \leq 0.0382$).

TABLE 2. Ten *Pasteurella haemolytica* biogroup variants that accounted for 87% of all isolates; although these data suggest temporal trends in relative prevalence among and within biogroups, such trends were independent of vaccine treatment.

Biogroup[b]	Total isolations	Prevalence[a] by month				
		Feb	Mar	Apr	May	Sep
2	179	0.83	0.77	0.73	0.83	1.00
16_ABE	56	0.50	0.47	0.47	0.17	0.10
1_A	25	0.10	0.20	0.30	0.04	0.03
7	23	0.20	0.07	0.30	0.13	0.03
U_ABL	22	0.27	0.13	0.10	0.00	0.00
10_A	22	0.03	0.13	0.00	0.30	0.14
2_CDS	21	0.10	0.20	0.37	0.00	0.03
2_CD	20	0.13	0.17	0.00	0.33	0.07
U_BELX	18	0.00	0.17	0.17	0.04	0.00
16_AE	17	0.17	0.10	0.17	0.04	0.00

[a] Calculated as isolations/bighorns sampled.

[b] Biogroup classifications followed criteria of Bisgaard and Mutters (1986), modified by CVTRC to allow negative variants in reactions to α-fucosidase (_A), maltose (_B), cellobiose (_C), salicin (_D), sorbitol (_E), β-galactosidase (_G), β-glucosidase (_L), esculin (_S), or xylose (_X).

Mean pretreatment titers for agglutinating antibody to *P. haemolytica* surface antigens ranged from ≤3.9 for serotype A1 to ≥9.3 for serotype T10 (Fig. 1B–D), but these also remained relatively stable in unvaccinated controls. Agglutinating antibody titers to serotype A1 surface antigens were elevated ($P = 0.0017$) in vaccinated bighorns beginning 1 wk after initial vaccination ($P = 0.0001$) and showed response patterns similar to those of neutralizing antibodies: mean responses peaked at 2 wk and titers remained elevated ≥6 but <8 wk in both dose groups ($P \leq 0.0036$) (Fig. 1B). Although elevation ($P = 0.0310$) of agglutinating antibody titers to serotype A2 was less dramatic, titers rose within 2 wk after initial vaccination ($P \leq 0.0018$) and remained elevated ≥4 but <8 wk in dose group 1 ($P \leq 0.0106$) and ≥8 but <29 wk in group 2 ($P \leq 0.0303$) (Fig. 1C); in addition, during wk 4 mean

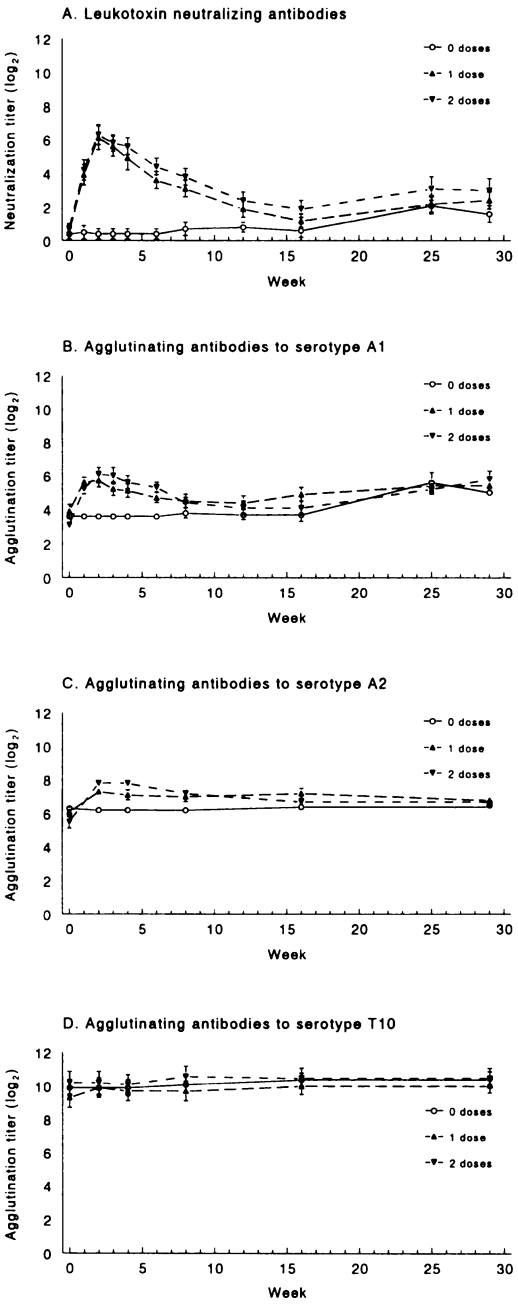

FIGURE 1. A. Vaccinated bighorns showed marked elevations in *P. haemolytica* leukotoxin neutralizing antibody titers as compared to unvaccinated controls. B–C. Titers of agglutinating antibody to *P. haemolytica* serotype A1 and A2 surface antigens were also elevated in vaccinated bighorns. D. Titers to *P. haemolytica* serotype T10 surface antigens were high in all treatment groups and were unaffected by vaccination. Points are mean observations; vertical lines are ±1 standard error of mean observations.

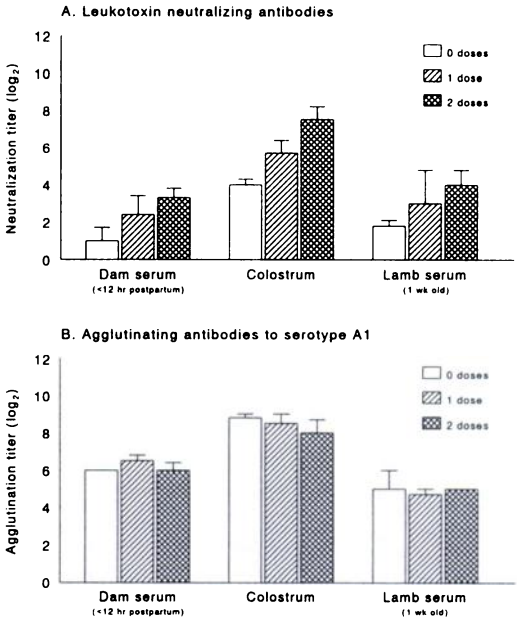

FIGURE 2. A. Vaccination of bighorn sheep 7 to 14 wk prior to parturition elevated leukotoxin neutralizing antibody titers in colostrum, but postpartum serum titers in vaccinated dams and their 1-wk-old lambs did not differ from controls. B. Serotype A1 surface antigen agglutinating antibody titers in colostrum and in postpartum serum from dams and lambs did not differ among vaccine dose groups. Bars are mean observations; vertical lines are +1 standard error of mean observations.

titers in group 2 were higher ($P = 0.0407$) than those in group 1. Titers of agglutinating antibody to *P. haemolytica* serotype T10 surface antigens were relatively high in all three groups and appeared unaffected by vaccination ($P = 0.4950$) (Fig. 1D). We also detected differences in agglutinating antibody titers to serotype A1 ($P = 0.0366$) and T10 ($P = 0.0001$) among age/sex strata. Differences appeared age-related, particularly for antibodies to serotype T10: the highest mean titers tended to occur in adult ewes and the lowest titers occurred in 9 mo old bighorns.

Antibodies to *P. haemolytica* leukotoxin and serotype A1 surface antigens were detected in colostrum and <12 hr postpartum serum from bighorn dams and in serum from their lambs (Fig. 2). Vaccination 7 to 14 wk prior to parturition elevated

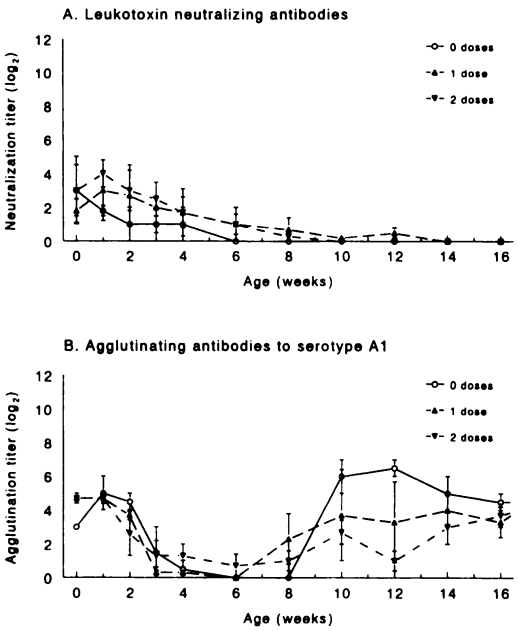

FIGURE 3. Neither (A) leukotoxin neutralizing nor (B) serotype A1 surface antigen agglutinating antibody titers differed through 16 wk of age among bighorn sheep lambs born to dams that had previously received 0, 1, or 2 vaccine doses. Although titers to both antigens declined steadily in lambs over the first 4 to 8 wk of life, only agglutinating antibody titers eventually returned to neonatal levels. Points are mean observations; vertical lines are ±1 standard error of mean observations.

leukotoxin neutralizing antibody titers in colostrum ($P = 0.0103$), but serum titers in vaccinated dams and their 1-wk-old lambs did not differ from controls ($P \geq 0.1713$) (Fig. 2A); small sample sizes may have precluded detection of additional differences, particularly among lambs. Serotype A1 surface antigen agglutinating antibody titers in colostrum, as well as in serum from dams and lambs, did not differ among vaccine dose groups ($P \geq 0.4053$) (Fig. 2B). Neither leukotoxin neutralizing nor serotype A1 agglutinating antibody titers differed ($P \geq 0.6475$) through 16 wk of age among lambs born to dams from different vaccine dose groups (Fig. 3). Both leukotoxin neutralizing and serotype A1 agglutinating antibody titers in bighorn lambs declined steadily in lambs over the first 4 to 8 wk after birth (Fig. 3). Mean agglutinating antibody titers subsequently began increasing when lambs were about 6 to 8 wk old and eventually returned to neonatal levels (Fig. 3B), but we detected no such active resurgence in bighorn lambs' leukotoxin neutralizing antibody titers (Fig. 3A).

DISCUSSION

Previous attempts to vaccinate bighorn sheep against pasteurellosis have yielded widely varied results. Although early studies of autogenous bacterins showed some promise of protection (Rufi, 1961; Post, 1962; Nash, 1972), these either failed in application (Howe, 1964) or they were never fully evaluated or incorporated into bighorn management programs. In more recent studies, neither an autogenous bacterin (Foreyt, 1992) nor prechallenge inoculation with cytotoxic *P. haemolytica* (Foreyt and Silflow, 1996) prevented captive bighorns from succumbing to pneumonic pasteurellosis. Moreover, use of a modified-live *P. haemolytica* A1 vaccine apparently caused pasteurellosis in previously healthy captive bighorns (Onderka et al., 1988). Direct comparisons of data among studies are confounded by differences in respective methodologies: our present study did not evaluate protection from experimental challenge, and previous studies either did not measure antibody responses to vaccination (Onderka et al., 1988; Foreyt, 1992; Foreyt and Silflow, 1996) or used serologic methods that preclude reliable comparisons (Rufi, 1961; Nash, 1972). Although protection from naturally occurring pasteurellosis was not credibly demonstrated in this study, our data at least showed no evidence that vaccination caused pasteurellosis or other health problems in bighorns.

Differences in agglutinating antibody titers to specific *P. haemolytica* serotypes most likely reflected differences in exposure to strains enzootic in our captive bighorn herd, but vaccination had no measurable effect on carriage of various enzootic *P. haemolytica* strains. Biotype T

isolates that reacted with serotype standards 3, 4 and 10 (=biogroup 2; Bisgaard and Mutters, 1986) were the most common *P. haemolytica* strains isolated from bighorns during this study (Table 2), and have been common for at least a decade in this captive herd (Miller et al., 1991b; Wild and Miller, 1991, 1994). We observed a strong association between exposure rates and high agglutinating antibody titers to serotype T10 surface antigens: biogroup 2 variants were isolated from ≥73% of bighorns at each sampling, and agglutinating titers to T10 were consistently highest across all treatment groups throughout our study. Differences in antigenicity among serotypes (Adlam, 1989) may also have contributed to observed differences among serotype-specific antibody titers. In contrast, exposure to enzootic *P. haemolytica* strains alone was apparently insufficient to stimulate leukotoxin neutralizing antibodies; alternatively, enzootic strains may not have been capable of producing leukotoxin.

Leukotoxin-neutralizing and serotype-specific agglutinating antibody responses made by bighorns receiving one or two vaccine doses were comparable to titers in free-ranging bighorns that survived naturally-occurring epizootics (M. W. Miller, unpubl. data). Titers also equaled or exceeded those associated with protection from experimental challenge in domestic sheep and cattle vaccinated with similar vaccines (A. Alexander, pers. comm.; Conlon et al., 1995). We believe that bighorn antibody responses measured here, combined with data from subsequent challenge trials (Kraabel et al., 1998), offer promise that the vaccine evaluated in our study may protect bighorn sheep from pneumonic pasteurellosis under natural conditions.

Bighorn responses to vaccination observed in this study suggest several potential applications in managing pasteurellosis in wild bighorns. Antibody levels in vaccinated bighorns rose rapidly and remained elevated for 12 to 16 wk (Fig. 1). Such responses could be beneficial to wild sheep vaccinated either annually or early in the course of a pneumonia epizootic. Perhaps of equal importance, trends in antibody levels detected in colostrum and neonatal lambs may reflect passive transfer of protective antibodies from vaccinated bighorn ewes to lambs similar to passive transfer from domestic ewes to lambs (Gilmour et al., 1980; Kiorpes et al., 1991), and from dairy cows to calves (Hodgins and Shewen, 1994). Because pasteurellosis in neonatal bighorn lambs may be the single most important factor hampering population recovery from an epizootic, vaccine-mediated protection could vastly diminish the long-term impacts of pasteurellosis on bighorn population performance.

Conferring protection to young bighorn lambs through ewe vaccination could be an effective means of reducing lamb mortality that is a common sequela of pasteurellosis epizootics in wild bighorn herds (Marsh, 1938; Onderka and Wishart, 1984; Festa-Bianchet, 1988; Foreyt, 1990). Our data suggest that ewe vaccination would not prevent lambs' colonization with *P. haemolytica* and that any conferred protection would likely be ephemeral at best. However, such protection could be sufficient to allow neonatal bighorn lambs to recover from pneumonic pasteurellosis and subsequently develop natural immunity to future infections (Donachie et al., 1986; Kiorpes et al., 1991; Hodgins and Shewen, 1994). Resurgence of agglutinating antibody titers in bighorn lambs beginning at 6 to 8 wk of age was most likely the product of active humoral responses to proliferation of enzootic *P. haemolytica* strains in their upper respiratory tracts; similar response patterns occur in domestic lambs (Gilmour et al., 1980; Kiorpes et al., 1991). Were lambs colonized with pathogenic *P. haemolytica,* passively transferred antibodies might aid in diminishing pulmonary damage (Jones et al., 1989) while lambs developed active immunity to both surface antigens and leukotoxin during their first 6 to 8 wk of life.

Our data demonstrate that this experimental *P. haemolytica* vaccine is safe and may stimulate antibody responses sufficient to confer protective immunity to bighorn sheep. Stimulation of dramatic antibody responses by a single vaccine dose greatly enhances prospects for its use in wild bighorns. Moreover, potential for delivery via oral carriers (Bowersock et al., 1994) or biodegradable implants makes application to free-ranging bighorn populations a tangible goal. Consequently, we believe further evaluation of this vaccine as a tool in preventing and managing pasteurellosis in bighorn sheep is warranted.

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