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ABSTRACT: The efficacy of a multivalent *Pasteurella haemolytica* vaccine (A1, A2, T10) in reducing morbidity and mortality associated with pneumonic pasteurellosis in bighorn sheep (*Ovis canadensis*) was examined. Fifteen captive bighorns were divided equally into three treatment groups based on vaccination status: control (no vaccination), one dose 10 days prior to challenge, or one or two doses 57 wk prior to challenge. At challenge, each bighorn received about 6.2×10^7 colony forming units of *P. haemolytica* (biotype T, serotype 10, biogroup 4_{CDS}, ribotype E_{CO}; "Alamosa Canyon" strain) suspension sprayed into the proximal trachea. Vaccination reduced ($P = 0.1$) mortality in bighorns vaccinated 10 days prior to challenge as compared to controls. Although mortality rates in bighorns vaccinated 57 wk prior to challenge did not differ from controls ($P = 0.26$), a trend in reduced mortality was apparent. Ranked cumulative postmortem scores based on observed gross lesions and bacteriology did not differ ($P \geq 0.14$) between vaccinated animals and control animals. Neutralizing antibody titers to *P. haemolytica* leukotoxin were elevated ($P = 0.003$) at challenge in bighorns vaccinated 10 days before challenge, and neutralizing titers in bighorns from both vaccinated groups were elevated at death ≤ 7 days after challenge ($P \leq 0.004$). In contrast, agglutinating antibody titers to *P. haemolytica* serotype A1 or T10 surface antigens did not differ between vaccinated and unvaccinated bighorns ($P \geq 0.19$). Based on these data, we believe that this experimental *P. haemolytica* vaccine is safe and can stimulate protective immunity from pneumonic pasteurellosis in bighorn sheep. Further evaluation of this vaccine as a tool in preventing and managing pasteurellosis in wild bighorn sheep appears warranted.

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

INTRODUCTION

Epidemics of respiratory disease have probably plagued bighorn sheep (*Ovis canadensis*) populations for at least a century (Buechner, 1960; Post, 1962). *Pasteurella* spp. are perhaps the most common pathogens isolated from bighorns during these epidemics (Potts, 1937; Marsh, 1938; Post, 1962; Onderka and Wishart, 1984; Festa-Bianchet, 1988; Miller et al., 1991). The inability to prevent or control mortality caused by pneumonic pasteurellosis represents a significant obstacle to successful long-term bighorn management (Onderka and Wishart, 1984; Festa-Bianchet, 1988; Hobbs and Miller, 1992).

Despite intensive study of the bighorn pneumonia complex, few tools for its management have emerged. Although vaccination has been suggested as a potential management tool for nearly four decades, previous attempts to vaccinate bighorn sheep against pasteurellosis have yielded largely discouraging results. Early studies of autogenous bacterins showed some promise of protection (Rufi, 1961; Post, 1962; Nash, 1972), but these vaccines either failed in application (Howe, 1964) or were never fully evaluated or incorporated into bighorn management programs. Other attempts to prevent pneumonia in bighorns using an autogenous bacterin or pre-challenge inoculation with cytotoxic *P. haemolytica* also failed (Foreyt, 1992; Foreyt

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and Silflow, 1996), and use of a modified-live *P. haemolytica* A1 vaccine apparently caused pasteurellosis in healthy bighorns (Onderka et al., 1988).

More recently, Miller et al. (1997) described a multivalent *P. haemolytica* vaccine (A1, A2, T10) that stimulated marked elevations in antibody titers to leukotoxin and surface antigens. The magnitude of antibody responses in vaccinated bighorns equaled or exceeded those associated with protection from experimental challenge in domestic sheep and cattle treated with similar vaccines (Miller et al., 1997). Here, we examined the efficacy of this multivalent vaccine in reducing morbidity and mortality in captive bighorn sheep challenged with a pathogenic strain of *P. haemolytica*.

MATERIALS AND METHODS

We used 17 captive Rocky Mountain bighorn sheep (*O. canadensis canadensis*) and four captive bighorn × mouflon f2 hybrid sheep (*O. canadensis canadensis* × *O. musimon*) in this experiment. All sheep were housed at the Colorado Division of Wildlife's (CDOW) Foothills Wildlife Research Facility (Fort Collins, Colorado, USA; 40°35'N, 105°10'W) throughout the study. Grass/alfalfa hay mix and a pelleted high-energy supplement were provided as prescribed under established feeding protocols for bighorn sheep in respective age/sex classes (Miller, 1990); fresh water and mineralized salt blocks were provided *ad libitum*. The resident bighorn herd was lungworm-free and had no previous evidence of infection with respiratory viruses (M. W. Miller, unpubl. data).

In evaluating vaccine efficacy, a repeatable representative model of natural pneumonic pasteurellosis in bighorn sheep was essential. Consequently, we conducted a pilot study to establish the effective minimum dose of *P. haemolytica* and optimal administration route needed to cause acute pneumonic pasteurellosis in bighorns. We used all four bighorn × mouflon hybrids and two bighorns in this pilot study; hybrid sheep were obtained from the Wyoming Game and Fish Department's Sybille Wildlife Research and Conservation Unit (SWRCU) (Wheatland, Wyoming, USA) in December 1995. Two or three individuals resided together in 100m² isolation pens throughout this study.

We used a field strain of *P. haemolytica* biotype T, serotype 10, biogroup 4_{CDS}, ribotype

E_{CO} ("Alamosa Canyon" strain) for challenge. This isolate was originally recovered from lung tissue collected in 1990 during a pasteurellosis epidemic in a free-ranging bighorn population in southcentral Colorado (M. W. Miller, unpubl. data) and had been passed <5 times on artificial media; previous *in vitro* evaluations of this isolate demonstrated marked leukotoxin production (Kraabel and Miller, 1997). Challenge doses were prepared by quick-thawing frozen *P. haemolytica* aliquots, placing them on 5% sheep blood agar plates (BBL Microbiology Systems, Becton Dickinson and Company, Cockeysville, Maryland, USA), and incubating them for 16 to 20 hr at 37 C. Isolated colonies were used to inoculate brain heart infusion broth (BHIB) (Difco Laboratories, Detroit, Michigan, USA); after 6 hr, this broth was diluted with fresh BHIB to an optical density at 525 nm (OD_{525}) of 0.1 and incubated until the OD_{525} reached 0.29 to 0.3. The culture was centrifuged at 4,000 × G for 15 min and resuspended in sterile phospate buffered saline (PBS) solution at estimated concentrations of 10^4, 10^6, 10^8 or 10^{10} colony forming units per ml (CFUs/ml). An aliquot of each resulting challenge dose level was plated, and doses were confirmed by counting colonies after 16 hr of incubation at 37 C.

We administered bacteria by sedating the sheep with 5 to 20 mg xylazine hydrochloride (HCL) (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) intravenously (IV) or intramuscularly (IM), opening their mouths, and visualizing the arytenoids with the aid of a laryngoscope. We initially administered the challenge dose by spraying culture solution onto the glottis (OP); subsequently, we delivered the bacteria into the proximal trachea (IT) (Table 1). Sedation was reversed with 30 to 50 mg yohimbine HCl (Wildlife Pharmaceuticals) IV immediately after challenge. One hybrid (MB4) was immobilized with a combination of 1.5 mg carfentanil HCl (Wildlife Pharmaceuticals), 90 mg ketamine HCl (Wildlife Pharmaceuticals), and 20 mg xylazine HCl (Wildlife Pharmaceuticals) IM for his second challenge; in this case, immobilization was reversed with 40 mg yohimbine HCl IV and 150 mg naltrexone HCl (Wildlife Pharmaceuticals), divided ¼ IV and ¾ subcutaneously.

Postchallenge, sheep were observed ≥3 times daily for signs of respiratory disease. In cases where the first selected dose/route combination failed to produce appreciable clinical signs or mortality in pilot animals ≤4 days postchallenge, those individuals were challenged again with a different dose/route combination (Table 1). Sheep that survived ≥4 days after a second challenge were sedated with 100

TABLE 1.   Chronological listing of challenge doses in colony forming units (CFUs) per ml of *Pasteurella haemolytica* and routes used in a pilot study determining optimal techniques for vaccine challenge in captive bighorn sheep.

Dose (CFUs)/ route[a]	Animal[b]	Outcome (duration)[c]/ postmortem score[d]
10^4/OP	MB-2	alive/rc
10^6/OP	MB-3	alive/rc
10^8/OP	MB-1	alive/rc
10^{10}/OP	MB-2	died (<39 hr)/71
10^8/IT	MB-3	died (<72 hr)/65
10^{10}/IT	MB-1	died (<15 hr)/69
10^6/IT	MB-4	alive/rc
10^7/IT	MB-4	alive/19
10^7/IT	B-L395	died (<60 hr)/65
10^6/IT	B-Q95	alive/2

[a] OP = oropharyngeal; IT = intratracheal.
[b] MB = mouflon × bighorn hybrid sheep; B = bighorn sheep.
[c] Alive = survived >96 hr postchallenge; died = died (hr) postchallenge; rc = rechallenged.
[d] Maximum postmortem score = 72; see text for scoring methods.

to 300 mg xylazine HCl IV or IM and euthanized by IV injection of 100 mg/kg pentobarbital sodium solution (Beuthanasia®-D, Schering-Plough Animal Health Corporation, Kenilworth, New Jersey, USA).

All sheep were necropsied immediately after death. Gross lesions were photographed and scored using a system adapted from Jones et al. (1989). Each animal received a cumulative postmortem score (maximum = 72) based on severity of gross lung lesions, gross evidence of other organ involvement, and *P. haemolytica* isolation from select tissues. At necropsy, we diagramed areas of lung involvement and calculated a lung lesion score by estimating the proportion of each lung lobe affected from both dorsal and ventral aspects. Each lobe's contribution to the total score was extrapolated from estimated percentages of total lung volume occupied by each lobe in a normal domestic sheep's lung. The mean ventral and dorsal pneumonic scores were divided by 2 to give a final lung lesion score (maximum = 50). In addition, the amount of fibrin present on the visceral or parietal pleura was scored as 0 (none), 1 (small amount), 3 (moderate amount), or 6 (substantial amount) (maximum = 6). We scored other organ involvement by adding 1 point each for subcutaneous, epicardial, renal capsular, splenic, hepatic, or parietal pleural hemorrhages (maximum = 6). Representative samples of lung (at lesion margins, where available), liver, spleen, kidney, and right

carpus were refrigerated and shipped overnight on ice packs to the Caine Veterinary Teaching and Research Center (CVTRC) (Caldwell, Idaho, USA) for culture and biotyping using methods described by Miller et al. (1997); two points were added for each site yielding *P. haemolytica* (maximum = 10).

We used 15 captive Rocky Mountain bighorn sheep in the vaccine challenge trial. Three or four individuals resided in 100m² isolation pens throughout the study. Pen assignments were made to accommodate social differences and thereby minimize stress on cohoused individuals, but treatment assignments were randomly distributed across pens.

Bighorns were subdivided into three groups of five based on vaccination status: 0 (control, no vaccination), 1 (vaccinated 10 days prior to challenge), or 2 (vaccinated 57 wks prior to challenge). Bighorns in groups 0 and 1 had not been vaccinated previously, and were assigned randomly to respective groups; bighorns in group 2 had received experimental vaccine in February 1995 during a previous study (Miller et al., 1997). The experimental *P. haemolytica* vaccine (Langford Laboratories, Incorporated, Guelph, Ontario, Canada; lot 940902) evaluated here was the same vaccine previously described by Miller et al. (1997).

After an acclimation period of 12 days in isolation pens, we aseptically injected 2 ml of vaccine IM into bighorns in treatment group 1; bighorns in groups 0 and 2 received 2 ml 0.9% saline, aseptically injected IM. Ten days after vaccination or saline injection, each bighorn received about 6.3 × 10⁷ CFUs of *P. haemolytica* (biotype T, serotype 10, biogroup 4 $_{CDS}$, ribotype E_{CO}). The challenge dose was prepared as described previously to a final concentration 1.25 × 10⁸/ml; sheep were inoculated with 500 μl of culture suspension diluted in 5 ml of PBS. To administer the challenge, we sedated each bighorn with 5 to 20 mg IV xylazine HCl, held open its mouth with a speculum, anesthetized the larynx with a 2% solution of lidocaine HCl (Sparhawk Laboratories, Kansas City, Kansas, USA), placed a spray nozzle into the glottis with aid of a laryngoscope, and sprayed diluted culture suspension in a fine mist into the proximal trachea. Sedation was reversed with 10 to 40 mg IV yohimbine HCl immediately after administration of the challenge dose.

This experiment was designed to evaluate the efficacy of vaccination in preventing or reducing pneumonic pasteurellosis. Although we anticipated some sheep would become clinically ill or die in response to challenge, our approach attempted to balance the need to evaluate clinical responses of challenged bighorns with the need to relieve pain and suffering in

affected animals. The health of bighorns was closely monitored by attending veterinarians ≥3 times daily (early morning, mid-day, and dusk) after challenge. Analgesic or antiinflammatory drugs were not provided to affected animals because they might have interfered with vaccine-mediated responses to challenge. Moreover, because the goal of this research was to determine whether the vaccine protected bighorns in the face of infection with *P. haemolytica*, we did not euthanize animals demonstrating respiratory signs unless they became recumbant, extremely depressed, or moribund. All animals that died or were euthanized during the experiment were necropsied to determine cause of death; surviving bighorns were euthanized with 100 mg/kg pentobarbital sodium solution (Beuthanasia® -D) 7 days after challenge and necropsied. All necropsies and sampling were performed immediately after death and carcasses scored as described above. Representative tissue samples from all major organ systems also were collected and placed in 10% neutral buffered formalin and submitted for histological evaluation (Wyoming State Veterinary Laboratory, Laramie, Wyoming, USA).

In addition to postmortem samples, we collected 10 to 12 ml of blood via jugular venipuncture for antibody measurements from each bighorn immediately prior to vaccination, challenge, and death; we also collected oropharyngeal and nasal swabs from each bighorn in conjuction with sample collections for serology. All blood samples were held 1 to 4 hr at 22 C, centrifuged, and serum collected. Serum was stored at −20 C until analyzed. Swabs were placed in transport tubes containing modified Cary and Blair medium (Port-A-Cul®, Becton Dickinson and Company) and shipped overnight on ice packs to CVTRC for culture and analysis. Measurement of leukotoxin neutralizing antibodies and agglutinating antibodies to serotype-specific surface antigens, as well as culture and biogrouping of *P. haemolytica* from nasal and oropharyngeal swabs and tissue samples, followed methods described by Miller et al. (1997); titers were expressed as $\log_2$.

In evaluating vaccine efficacy, we compared (1) mortality rates, (2) postmortem scores, (3) serum neutralizing antibody titers to *P. haemolytica* leukotoxin, and (4) serum antibody titers to *P. haemolytica* surface antigen; in all cases, we compared each vaccine group to controls but did not compare between vaccine groups. Mortality rates were compared using Fisher's exact test (Mielke and Berry, 1992). Postmortem scores among groups were compared using Wilcoxon rank sum test with ties (SAS Institute, Inc., 1989). We analyzed serologic data using least squares analysis of variance (ANOVA) for general linear models (SAS Institute, Inc., 1989). Antibody responses were analyzed with ANOVA for a randomized complete block design with a repeated measures structure, with vaccine treatment group (0, 1, 2) as the sole main effect and time treated as a within subject effect (Morrison, 1976); we used least squares means for pairwise comparisons of antibody titers between each vaccine group and controls at vaccination, challenge, and postmortem. We used α = 0.1 for all statistical comparisons. Our challenge study was designed with sample sizes sufficient to offer power of ≥0.63 in detecting ≥4-fold differences in mortality between treatment and control groups.

RESULTS

Spraying about 10^4, 10^6, or 10^8 CFUs of *P. haemolytica* into the oropharyngeal region of bighorn × mouflon hybrid sheep failed to produce acute pneumonic pasteurellosis, and none of the sheep died (Table 1); however, about 10^{10} CFUs sprayed OP caused death in one hybrid <39 hr later. Delivered IT, about 10^6 CFUs failed to cause clinical signs or significant lesions, but 10^8 or 10^{10} CFUs IT caused fatal pneumonia and/or systemic disease within 72 hr (Table 1) in hybrids. Dosed at about 10^7 CFUs IT, the challenge strain caused severe pneumonia in a hybrid and fatal pneumonia and systemic disease in a bighorn (Table 1).

Vaccinating bighorns 10 days prior to challenge reduced ($P = 0.1$) mortality (1/5) as compared to those for controls (4/5); although mortality (2/5) in bighorns vaccinated 57 wk prior to challenge did not differ from controls ($P = 0.26$), a trend in reduced mortality was apparent (Fig. 1). Clinical signs were apparent in some animals <18 hr after challenge; eleven of the 15 bighorns, including all five controls, showed at least moderate signs of respiratory disease (depression, shallow or labored respiration, anorexia) ≤72 hr after challenge. Four of five controls died about 30 to 72 hr postchallenge; three vaccinated bighorns succumbed about 84 to 120 hr postchallenge. At necropsy, we observed evidence of acute pneumonia in all 15 big-
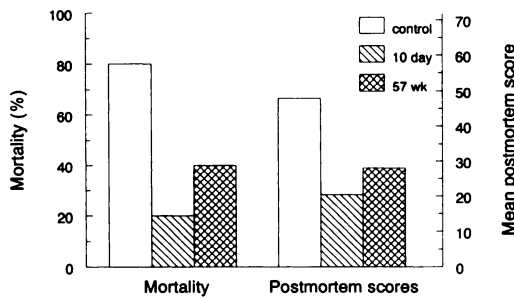
FIGURE 1.   Vaccination reduced bighorns' susceptibility to fatal pasteurellosis resulting from experimental challenge. Mortality was lower (P = 0.1) in bighorns vaccinated 10 days before challenge; mortality in bighorns vaccinated 57 wks prior to challenge did not differ from controls (P = 0.26), but a reduced trend was apparent. Similarly, lesions tended to be less severe in vaccinated animals, although ranked postmortem scores did not differ between controls and 10 day vaccinates (P = 0.14) or 57 wk vaccinates (P = 0.22).

horns. Lung damage and other lesions tended to be less severe in vaccinated animals (Fig. 1), but ranked postmortem scores did not differ between controls and 10 day vaccinates (P = 0.14) or 57 wk vaccinates (P = 0.22).

Leukotoxin neutralizing antibody titers were elevated (P = 0.003) at challenge in bighorns vaccinated 10 days previously as compared to titers in controls, and in both 10 day and 57 wk vaccinates at postmortem ≤7 days after challenge (P ≤ 0.004) (Fig. 2A). In contrast, titers of agglutinating antibody to *P. haemolytica* serotype A1 or T10 surface antigens did not differ between vaccinated and unvaccinated bighorns before, at, or after challenge (P ≥ 0.19) (Fig. 2B–C). All seven bighorns that succumbed had postchallenge leukotoxin neutralizing antibody titers ≤4.0; seven of eight challenge survivors had neutralizing titers ≥3.5 (Fig. 3).

We recovered *P. haemolytica* from all captive bighorns prior to challenge; in all, fourteen distinguishable biogroups were identified among 54 isolates. The challenge strain of *P. haemolytica* (biogroup 4-CDS) was not recovered from oropharyngeal or nasal swabs from any of the study bighorns at vaccination or at chal-
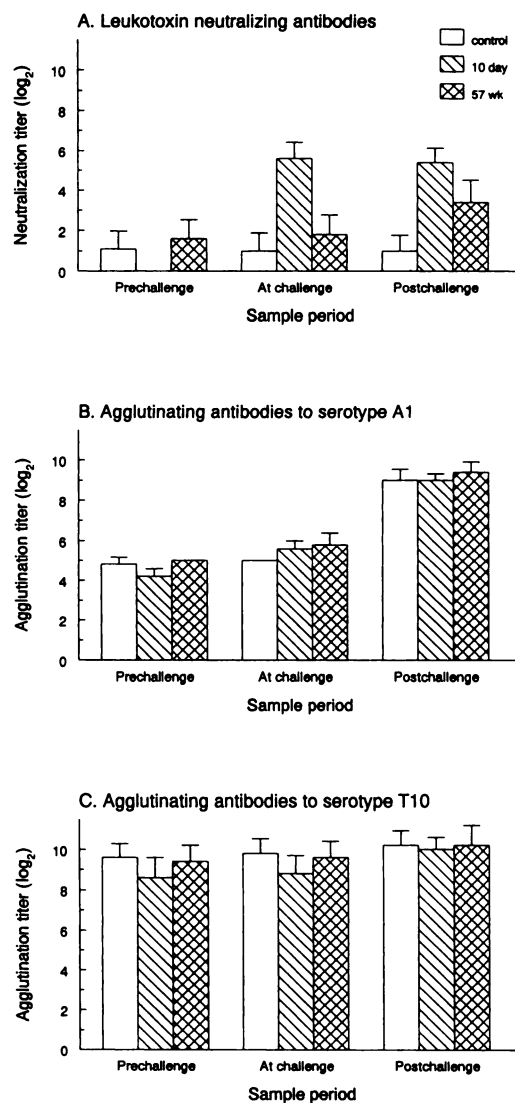

FIGURE 2.   Vaccination stimulated both leukotoxin neutralizing and agglutinating antibody responses in bighorns. Leukotoxin neutralizing antibody titers (A) were elevated (P = 0.003) at challenge in bighorns vaccinated 10 days previously, and in both 10 day and 57 wk vaccinates at postmortem ≤7 days after challenge (P ≤ 0.004). In contrast, titers of agglutinating antibody to *P. haemolytica* serotype A1 (B) or T10 (C) surface antigens did not differ between vaccinated and unvaccinated bighorns (P ≥ 0.19). Bars are mean observations; vertical lines are +1 standard error of mean observations.
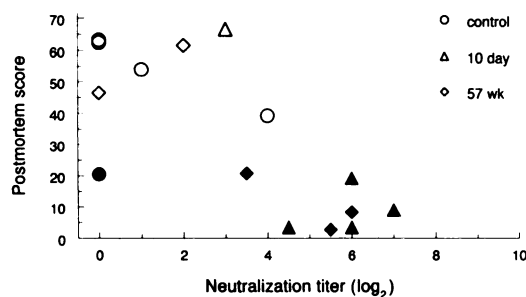
FIGURE 3.   Vaccine-stimulated humoral immune responses were associated with reduced mortality in bighorn sheep exposed to pathogenic *Pasteurella haemolytica*. All seven bighorns that succumbed to challenge (open symbols) had postchallenge leukotoxin neutralizing antibody titers ≤4.0; seven of eight challenge survivors (solid symbols) had neutralizing titers ≥3.5.

lenge, but was recovered from lung tissue or oropharyngeal swabs from all bighorns postmortem. In addition, this isolate was recovered from liver (6/7), kidney (4/7), spleen (4/7), and joint (2/7) samples from bighorns that succumbed to pasteurellosis; with the exception of one isolation from kidney, only lung or oropharyngeal samples from surviving bighorns yielded challenge strain postmortem. Three other distinguishable *P. haemolytica* biogroups were infrequently isolated from lung tissues postmortem. Microscopic examination of lung lesions confirmed necrotizing fibrinopurulent bronchopneumonia in all bighorns.

DISCUSSION

Our pilot study revealed that a moderate dose of *P. haemolytica* sprayed directly into the proximal trachea was an effective and reliable method for causing acute pneumonic pasteurellosis in bighorn and bighorn × mouflon hybrid sheep (Table 1). About 10^7 CFUs of *P. haemolytica* (biotype T, serotype 10, biogroup 4$_{CDS}$, ribotype E$_{CO}$, "Alamosa Canyon" strain) were sufficient to produce clinical disease and lesions in unvaccinated sheep that closely resembled those reported during naturally-occurring pasteurellosis epidemics in free-ranging bighorns (Onderka and

Wishart, 1984; Spraker et al., 1984). In vitro leukotoxicity of our challenge isolate was comparable to at least one isolate used in previous *P. haemolytica* challenge studies in bighorn sheep (Kraabel and Miller, 1997). However, most earlier experiments used IT inoculation doses of 10^9 to 10^{12} CFUs (Onderka et al., 1988; Foreyt et al., 1994; Foreyt and Silflow, 1996); although challenge doses varied widely among those studies, they consistently caused peracute to acute fatal bronchopneumonia resembling results we obtained using doses $\geq 10^8$ CFU. Two of these studies were designed to answer questions about the potential pathogenicity of *P. haemolytica* isolates in bighorn sheep, and none reported data on minimum effective challenge doses. By design, the challenge dose and route used to evaluate vaccine efficacy here were more severe than natural exposure, but we believe they afforded a reasonable basis for extrapolation to natural conditions.

Despite the severity of challenge, the multivalent *P. haemolytica* vaccine reduced mortality in bighorn sheep exposed to this pathogenic field strain of *P. haemolytica*. In all, 70% of vaccinated bighorns survived challenge, compared to just 20% of controls. All surviving bighorns showed some evidence of pneumonia regardless of vaccination status (Fig. 3); however, based on clinical and postmortem observations we believe that those individuals would likely have continued to recover unaided. The magnitude of mortality reduction in vaccinated bighorns was comparable to protection conferred by similar vaccines in domestic sheep and cattle (Alexander et al., 1995; Conlon et al., 1995). Based on postmortem scores, challenge produced relatively severe disease in vaccinated bighorns compared to scores reported in domestic sheep; we believe this is most likely a reflection of species differences in susceptibility to pasteurellosis (Onderka et al., 1988; Silflow et al., 1989). Vaccination had no measurable effect on carriage of various *P. haemolytica* strains endemic in captive bighorns, and clearly

did not prevent bighorns from being colonized with the challenge strain.

Although the precise mechanisms for vaccine protection were not studied here, bighorn survival appeared strongly correlated to the magnitude of leukotoxin neutralizing antibody titers measured postchallenge (Fig. 3). Agglutinating antibody titers to *P. haemolytica* surface antigens alone were clearly insufficient to protect bighorns from experimental challenge. Titers to serotype T10 surface antigens were relatively high throughout the study across all treatment groups (Fig. 2C), probably reflecting previous oropharyngeal exposure to nonpathogenic *P. haemolytica* strains endemic in this captive population (Miller et al., 1997). Similarly, modest prechallenge serotype A1 agglutinating titers rose in all three groups after challenge (Fig. 2B), but those elevated titers failed to protect controls from pasteurellosis. In contrast, vaccination stimulated marked elevation of leukotoxin neutralizing antibody titers in previously unvaccinated bighorns (Fig. 2A). Perhaps more importantly, challenge apparently stimulated anamnestic responses in previously vaccinated bighorns (Fig. 2A). Postchallenge leukotoxin neutralizing titers in seven of eight surviving bighorns approximated titers in free-ranging bighorns that survived naturally-occurring epidemics (M. W. Miller, unpubl. data), and also approximated titers in domestic sheep and cattle protected from experimental challenges (A. Alexander, pers. commun.; Conlon et al., 1995). Of the parameters we measured, leukotoxin neutralizing antibody titers seemed most useful in predicting the outcome of experimental challenge with pathogenic *P. haemolytica*. These observations are consistent with studies of immunity to pasteurellosis in domestic ruminants (Donachie, 1995; Shewen, 1995).

Responses to vaccination observed in this and a previous study (Miller et al., 1997) suggest several potential applications in managing pasteurellosis in wild bighorns. Because antibody titers in vaccinated bighorns rise rapidly and remain elevated for 12 to 16 wk (Miller et al., 1997), there may be benefits to vaccinating wild sheep early in the course of a pneumonia epidemic. Perhaps of equal importance, the trend toward reduced mortality in bighorns vaccinated >1 yr prior to challenge demonstrates the potential utility of annual vaccination to prevent epidemics in some management situations. Stimulation of dramatic antibody responses by a single vaccination greatly enhances prospects for its use in wild bighorns.

Although bighorn pasteurellosis displays several epidemiological features that might favor vaccination as an effective control strategy (Barlow, 1996), the efficacy of vaccination in long-term management of bighorn populations remains untested. Because pasteurellosis epidemics in free-ranging populations are sometimes complicated by infections with respiratory viruses, chlamydia, or parasitic lungworms, vaccination could prove less effective under field conditions. Variable pathogenicity among *P. haemolytica* strains (Miller et al., 1991; Miller et al., 1995; Kraabel and Miller, 1997) could also affect vaccine efficacy. Moreover, because pasteurellosis among lambs born into herds with immune carriers can severely impair recruitment after an epidemic, we recognize that improving individual bighorn survival during an epidemic may not necessarily lead to improved population performance or survival thereafter. However, our data suggest that the multivalent *P. haemolytica* vaccine used here is safe and stimulates protective immunity in bighorn sheep. Consequently, we believe field evaluation of this vaccine through carefully designed management experiments in free-ranging bighorn sheep is warranted.

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