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Authors: Ward, A. C. S., Hunter, D. L., Rudolph, K. M., DeLong, W. J., Bulgin, J. M., et al.

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IMMUNOLOGIC RESPONSES OF DOMESTIC AND BIGHORN SHEEP TO A MULTIVALENT *PASTEURELLA HAEMOLYTICA* VACCINE

A. C. S. Ward,[1,5] D. L. Hunter,[2] K. M. Rudolph,[2] W. J. DeLong,[1] J. M. Bulgin,[1] L. M. Cowan,[1] H. J. McNeil,[3] and M. W. Miller[4]

[1] University of Idaho, Caine Veterinary Teaching and Research Center, 1020 East Homedale Road, Caldwell, Idaho 83605-8098, USA
[2] Idaho Department of Fish and Game, 16569 South Tenth Street, Caldwell, Idaho 83605, USA
[3] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G2W1, Canada
[4] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA
[5] Corresponding author (e-mail address award@uidaho.edu)

ABSTRACT: The efficacy of a *Pasteurella haemolytica* vaccine (serotypes A1, A2, and T10) to induce humoral antibodies and alter colonization of the upper respiratory tract by related *P. haemolytica* spp. strains was evaluated in 10 bighorn (*Ovis canadensis canadensis*) and 10 domestic (*Ovis aries*) sheep. Sheep of each species were divided into five pairs based on age and history of respiratory disease. One sheep in each pair was vaccinated twice 2 wk apart with 2 ml of vaccine (VAC group) and the remaining animals (NV group) were injected with 2 ml of sterile saline. Mild, transient lameness was the only observed adverse effect. Blood sera from the sheep were tested for agglutinating antibodies against whole cells of A1, A2, and T10 and for leukotoxin neutralizing antibodies. Antibody titers were expressed as the reciprocal $\log_2$ of the highest reactive dilutions. Domestic sheep >1-yr-old and two bighorn sheep with a history of A1 infection had higher titers throughout the study against A1 cells than domestic sheep <1-yr-old and bighorns without a history of A1 infection. Both domestic and bighorn sheep had $\log_2$ titers of 8 to 12 against A2 cells and 6 to 12 against T10 cells during this time. Bighorn sheep in the VAC group had 2 to 32 fold titer increases for A1 cells by 2 wk post-vaccination (PV) compared to 0 to 2 fold increases in VAC domestic sheep. Two to 16 and 0 to 8 fold increases in antibodies titers to A2 and T10 cells, respectively, were detected in sera of both VAC groups. Sera of bighorn sheep with a history of respiratory disease and all domestic sheep had $\log_2$ leukotoxin neutralizing antibody titers of 4 to 14 in contrast to ≤2 in sera of bighorn sheep without a history of respiratory disease. Neutralizing antibody titers of two bighorns without a history of respiratory disease in the VAC group increased from $\log_2$ 0 to 5 in one and from 0 to 9 in the other 2 wk PV. Antibody increases in these animals were no longer evident at 16 wk PV while titers of animals with histories of disease remained relatively stable. The types and numbers of *Pasteurella* spp. isolated from nasal and pharyngeal swabs varied throughout the study without conclusive evidence of suppression of colonization. Although the animals were not experimentally challenged to determine the efficacy of the vaccine, one VAC and one NV bighorn sheep died following introduction of an A2 *P. haemolytica* strain when leukotoxin neutralizing antibodies had returned to pre-vaccination levels. This vaccine appeared to be safe for use in bighorn sheep and stimulated moderate but transient increases in antibody levels which should provide some protection against naturally occurring disease. A vaccine which would induce production of high and maintained antibodies against multiple strains of *P. haemolytica* would be valuable for use in bighorn sheep maintained in captivity or when captured for relocation.

Key words: Bighorn sheep, domestic sheep, immunization, *Ovis aries, Ovis canadensis canadensis, Pasteurella haemolytica* vaccine.

INTRODUCTION

Pneumonia associated with *Pasteurella haemolytica* is recognized in both domestic sheep (*Ovis aries*) and bighorn sheep (*Ovis canadensis canadensis*) (Onderka and Wishart, 1984; Gilmour and Gilmour, 1989; Ward et al., 1992). Pasteurellosis is responsible for considerable economic loss in domestic sheep and high morbidity and mortality in bighorn sheep populations (Onderka and Wishart, 1984; Salman et al., 1988). Pneumonic pasteurellosis in free-ranging bighorn sheep has been incriminated as the cause of approximately 75% reduction of some populations (Onderka and Wishart, 1984; Spraker et al., 1984; Coggins, 1988). Lamb mortality following these episodes has been reported

TABLE 1. Pairing and random treatment assignments for domestic and bighorn sheep included in immunization trials with experimental *Pasteurella haemolytica* toxoid vaccine.

Age	Species	Sex[a]	Health[b] history	Treatment group[c] and animal identification	
				NV	VAC
<1 year	bighorn	F, M	H, H	1	6
	domestic	F, F	H, H	20	21
		F, F	H, H	22	84
>1 year	bighorn	MC, M	P, P	2	7
		F, F	H, H	4	9
		M, F	P, H	5	10
		M, M	P, P	18	17
	domestic	F, F	H, H	144	C35
		F, F	H, H	331	116
		F, F	H, H	335	182

[a] F = female, M = male, MC = male castrate.
[b] H = healthy (i.e., no history of pneumonia), P = history of pneumonia.
[c] NV = animals were non-vaccinated, VAC = animals were vaccinated; numbers in each column are animal identification numbers.

to be nearly 100% (Onderka and Wishart, 1984; Spraker et al., 1984; Coggins, 1988). The possibility of preventing these episodes in bighorn sheep by immunization has been considered; however, previous immunization trials have not been encouraging (Onderka et al., 1988; Foreyt, 1992; Foreyt and Silflow, 1996). Serum antibody responses of bighorn sheep were not reported in those reports.

Pasteurella haemolytica biotype A serotype 2 (A2) is most commonly incriminated as the cause of pneumonia in domestic lambs during the first 2 mo after birth (Smith, 1961; Gilmour and Gilmour, 1989). Septicemic and pneumonic forms of pasteurellosis most common in domestic lambs at the time of weaning and movement to feed lots is caused by strains of *P. haemolytica* biotype T (Smith, 1961), for which a name change to *P. trehalosi* has been suggested (Sneath and Stevens, 1990). Prevention of these disease syndromes by immunization of domestic sheep has been attempted with varied results. Up to 98% protection resulting from vaccination has been reported (Sutherland et al., 1988) and serum antibody levels correlated with resistance to experimental challenge (Jones et al., 1989).

The objectives of this study were to determine if bighorn sheep could produce humoral antibody responses to specific antigens present in an experimental *P. haemolytica* vaccine, to quantitate the responses, and to compare responses of bighorn with those of domestic sheep. In addition bacterial cultures were conducted to determine if vaccination would influence *Pasteurella* spp. populations on the mucosa of the upper respiratory tract.

MATERIALS AND METHODS

Ten bighorn sheep raised in captivity were included in this study (Table 1). The sheep were assigned to five pairs based on age and past disease history. Two sheep, a female (#1) and a male (#6) with no history of respiratory disease, were <1-yr-old. Eight were >1-yr-old: two males (#2 and #7) which had respiratory disease following contact with domestic sheep 2 yr earlier; two females (#4 and #9) with no history of respiratory disease; two males (#17 and #18) which had been taken by caesarian section and had clinical pneumonia as lambs (Jaworski et al., 1993); and a male which had symptoms of respiratory disease as a lamb (#5) and a female with no history of respiratory disease (#10). Bighorns were assigned to pairs in January following rut and before it was known that ewes #9 and #10 were pregnant. Animals #2 and #7 were separated from the other bighorn sheep throughout the study due to pre-

vious contact with domestic sheep and disease history. The other bighorn sheep were intermingled during the study period. Ten domestic female sheep, four <1-yr and six >1-yr-old, with no history of disease were divided into age matched pairs for inclusion in this study. Bighorn and domestic sheep were initially penned separately at the Idaho Department of Fish and Game Wildlife Health Laboratory and the University of Idaho, Caine Veterinary Teaching and Research Center facilities, respectively, near Caldwell (Idaho; 43°60′N, 116°50′W). Bighorn sheep (#2 and #7) were penned with domestic sheep 8 wk after the initiation of the vaccination study for continuation of a separate experiment (data not included in this report). All sheep were provided free access to water and mixed grass and alfalfa hay.

One sheep in each pair was arbitrarily assigned to a nonvaccinated (NV) control group, and the other to a vaccinated (VAC) group. Each sheep in the VAC group was injected intramuscularly (IM) with 2.0 ml of an experimental *P. haemolytica* vaccine containing A1, A2, and T10 components including leukotoxoid (Langford Laboratories, Inc., Guelph, Ontario, Canada) as described previously (Miller et al., 1997). An initial dose was injected on 16 February (day 0) and a booster 1 March, 1995. Each sheep in the NV groups was injected IM with 2.0 ml of sterile saline (8.5% NaCl) on the same dates. All animals were observed daily for a week after each injection for any signs of adverse effects from the vaccination. Any animals observed during the study to have evidence of respiratory disease including harsh coughing, purulent nasal exudate, and depression were treated with antibiotics as follows: 1.25 mg/kg body weight amoxicillin (SmithKline Beecham Animal Health, West Chester, Pennsylvania, USA), each day for 3 days supplemented with 2 mg/kg gentamicin (SmithKline Beecham Animal Health) on day 4 and given alone for three additional days. Necropsies were conducted on animals that died.

Blood samples were collected from each animal at −2 wk, day 0, and 1, 2, 3, 4, 7, 11, 15, 18, 22, 26, and 30 wks following the first vaccine dose. Blood samples were allowed to set at approximately 22 C until clotted, they were then centrifuged, the serum drawn off, aliquoted and frozen at −20 C until tests were conducted to determine antibody titers.

Serum antibody titers against A1, A2, and T10 antigens were conducted using a direct agglutination procedure (Shewen and Wilkie, 1982; Miller et al., 1997). Washed formalized cells of each bacterial strain included in the vaccine served as the antigens. Two-fold dilutions were made for each serum sample and titers were expressed as the reciprocal of $\log_2$. Titrations were conducted to $\log_2$ of 12 for agglutinating antibodies to A2 and T10; titrations for the agglutinating antibodies for A1 were carried out to their $\log_2$ end point. Leukotoxin neutralizing antibodies were measured with a colorimetric assay using 2 fold dilutions of the sera incubated with a constant concentration of leukotoxin (Shewen and Wilkie, 1988; Miller et al., 1997). An aliquot of each serum/leukotoxin mixture was subsequently added to constant quantities of bovine leukemia-derived (BL-3 line) B cells (American Type Culture Collection, Rockville, Maryland, USA). End points were expressed as the reciprocal $\log_2$ of the highest serum dilution which neutralized ≥50% toxicity compared to control tests.

Swab samples were collected from the nasal passage and the oropharyngeal region on the same days as blood was collected. Nasal samples were collected using rayon tipped swabs supplied with Amies transport medium with charcoal (Precision Dynamics Corporation, San Fernando, California, USA). Oropharyngeal samples were collected using Accu-Culshure™ (Accu-Med Corporation, Pleasantville, New York, USA) collection and transport systems. Samples collected from necropsied animals including heart blood, lung tissue, nasal and pharyngeal swabs and a uterine swab sample from a ewe which died during parturition. Samples were inoculated onto culture media within 1 hr of collection. Culture media included Columbia blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) supplemented with 5% ovine blood (CBA), and a Columbia blood agar with 5% bovine blood plus antibiotics (CBAA) selective for Pasteurellaceae (Ward et al., 1986). Inoculated media were incubated at 35 C in atmosphere with 10% added CO_2 and examined for bacterial colonies characteristic of *Pasteurella* spp. The numbers of each colony type were estimated using 1+, 2+, 3+, and 4+ for 1–9, 10–49, 50–99, and >100 colony forming units (CFU) respectively. A representative of each colony type was selected for further evaluation using essential tests of Bisgaard and Mutters (1986) as modified by Jaworski et al. (1998) to assign isolates to species and biovariant groups. Isolates which were identified in biovariant groups 1 or 2 were serotyped (Frank and Wessman, 1978) to monitor for suppression of *Pasteurella* spp. shedding in response to vaccination, since *P. haemolytica* A1 and A2 strains are in biogroup 1 and the T10 strain is in biogroup 2. Restriction enzyme analysis (REA) was conducted by established procedures (Jaworski et al., 1993) on isolates from animals with evidence of respiratory disease and animals which died.

Due to the limited number of bighorn sheep available for this study and multiple variables relative to age, disease history, and treatment meaningful statistical evaluations could not be conducted. However, observed variances were noted and will be discussed.

RESULTS

Bighorn and domestic sheep were slightly lame in the injected leg for 2 to 3 days but no other adverse reactions were detected relative to the vaccination. Serum antibody titers against A1, A2, T10, and leukotoxin components of the experimental vaccine are presented in Figures 1–4.

Agglutinating antibody titers against the A1 component are presented in Figure 1. Domestic sheep in the NV group had $\log_2$ titers ranging from 5 to 13 at the first test period (Fig. 1A). These titers remained relatively stable throughout the test period. However one domestic ewe (#331) in the NV group had an increase from $\log_2$ of 13 to 16 at 8 wk post-vaccination (PV) and then returned to previous levels by week 16. Two other sheep in this group had smaller increases. Domestic sheep in the VAC group had titers similar to those in the NV group (Fig. 1B). The two sheep <1 yr of age in each group had lower titers than older animals. One bighorn sheep in each of the NV and VAC groups (#2 and #7 respectively) had much higher titers to the A1 antigen than the other four animals in their respective groups (Fig. 1C, D). These two animals and #10, #17 and #18 had previous histories of respiratory disease (Table 1). Increases in PV titers ranged from 2 to 8 fold in other animals with the greatest increase evident in the sera of the <1-yr-old animal.

Results of tests for agglutinating titers against the A2 antigen are shown (Fig. 2). All domestic sheep in the NV group had $\log_2$ titers of ≥12 throughout the test period and those in the VAC group had $\log_2$ titers of ≥10 (Fig. 2A and 2B). Bighorns #2 and #7 had $\log_2$ titers of 10 to 12 (Fig. 2C and 2D). Two to 8 fold increases were evident for all the bighorns in the VAC

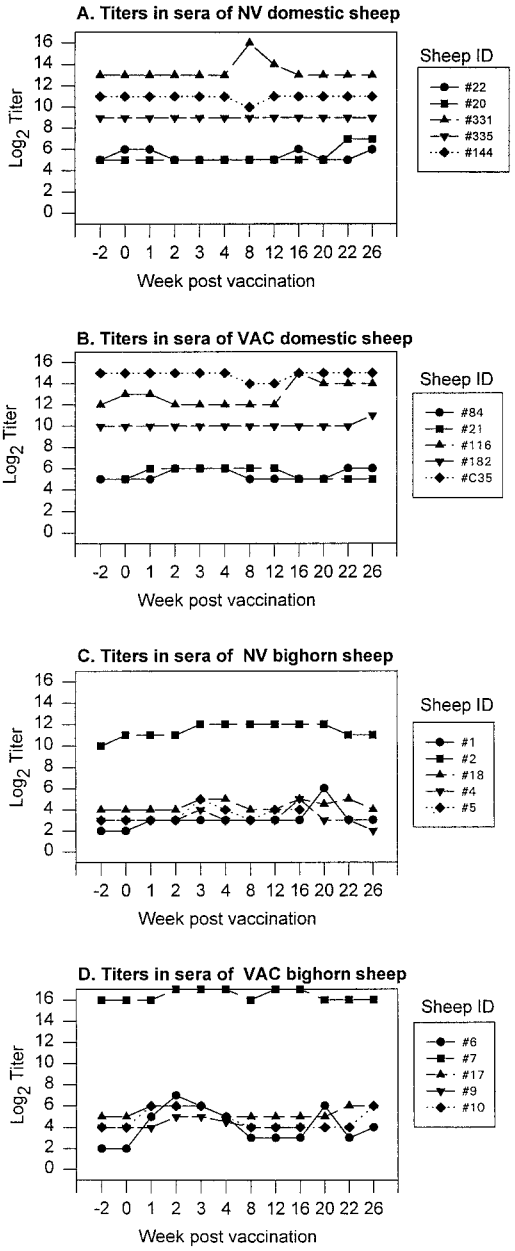

FIGURE 1. $\log_2$ agglutination antibody titers to serotype A1 *Pasteurella haemolytica* antigens. A. Non-vaccinated (NV) domestic sheep. B. Vaccinated (VAC) domestic sheep. C. NV bighorn sheep. D. VAC bighorn sheep. Sheep in VAC groups were given their first and second vaccine injections the same days samples were collected at weeks 0 and 2, respectively.

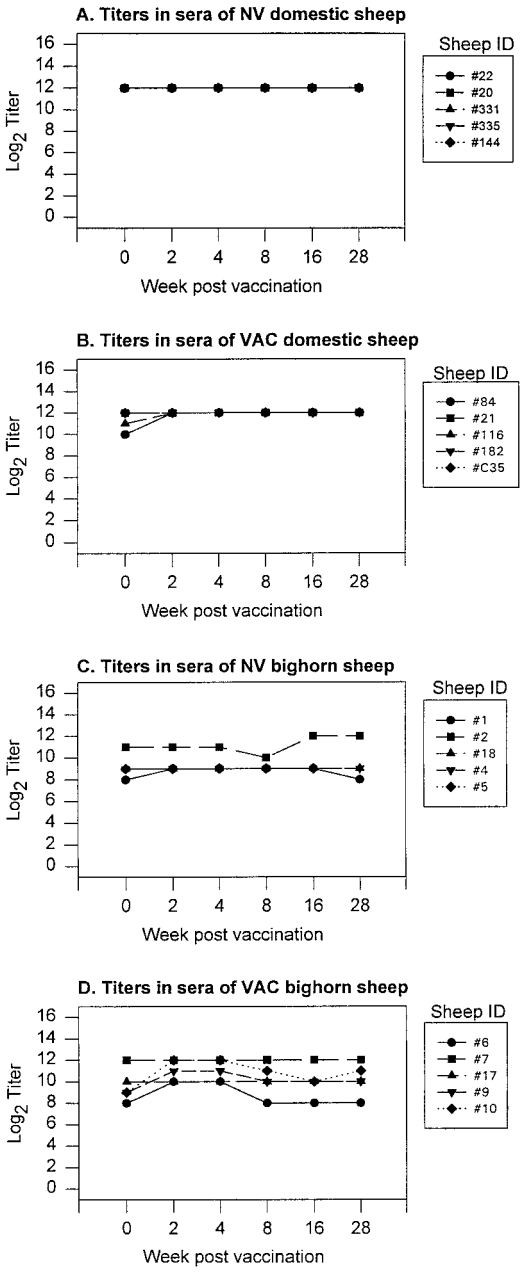

FIGURE 2. Log_2 agglutination antibody titers to serotype A2 *P. haemolytica* antigens. A. Non-vaccinated (NV) domestic sheep. B. Vaccinated (VAC) domestic sheep. C. NV bighorn sheep. D. VAC bighorn sheep. Sheep in VAC groups were given their first and second vaccine injections the same days samples were collected at weeks 0 and 2, respectively.

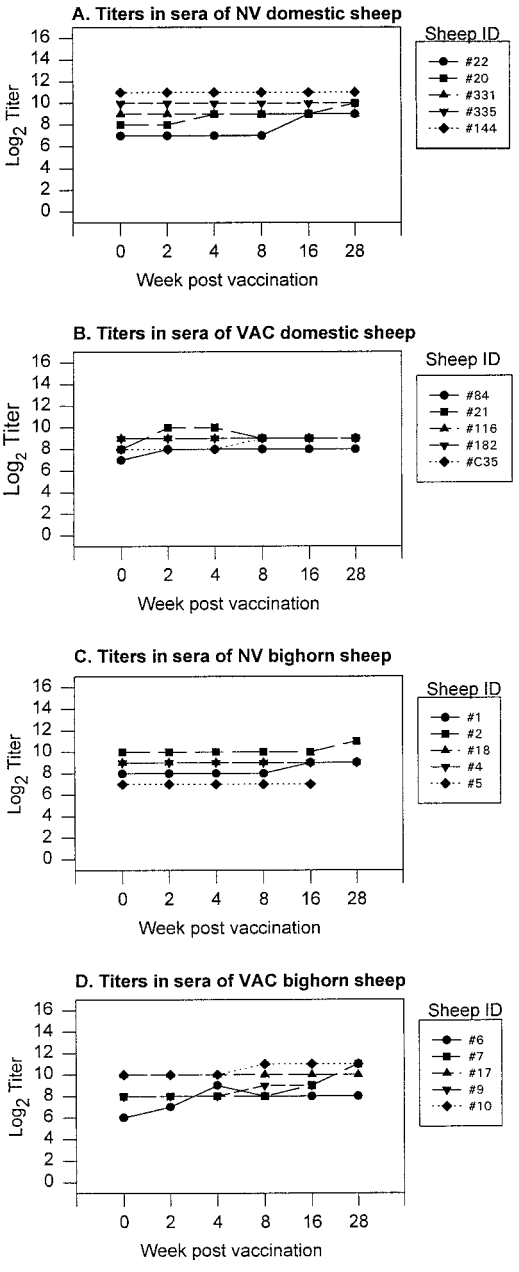

FIGURE 3. Log_2 agglutination antibody titers to serotype T10 *P. haemolytica* antigens. A. Non-vaccinated (NV) domestic sheep. B. Vaccinated (VAC) domestic sheep. C. NV bighorn sheep. D. VAC bighorn sheep. Sheep in VAC groups were given their first and second vaccine injections the same days samples were collected at weeks 0 and 2, respectively.

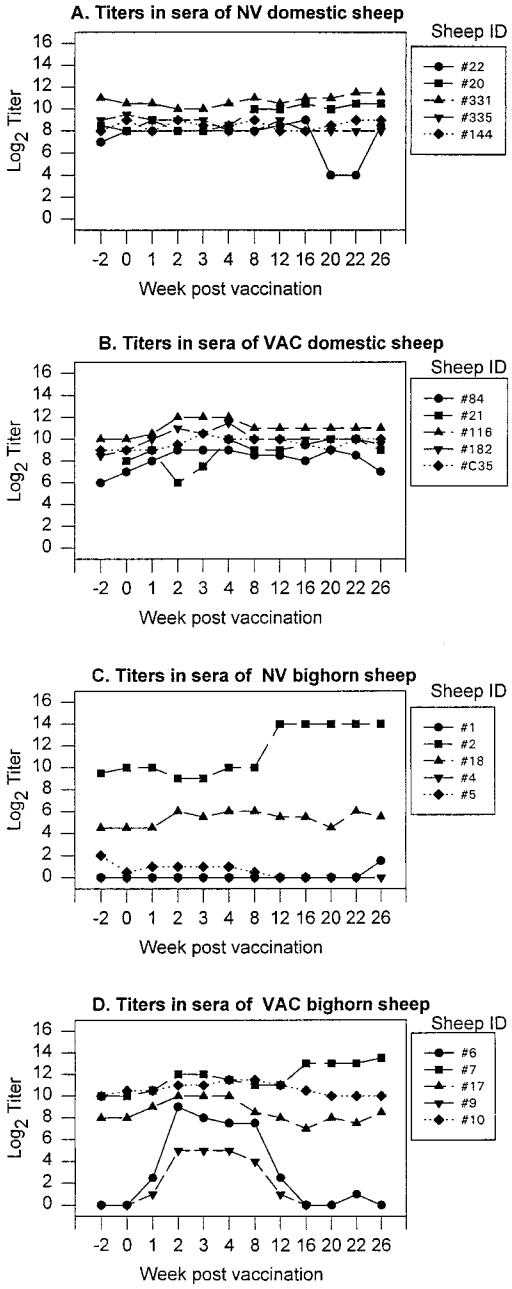

FIGURE 4. Serum neutralizing antibody titers to *Pasteurella haemolytica* leukotoxin. A. Non-vaccinated (NV) domestic sheep. B. Vaccinated (VAC) domestic sheep. C. NV bighorn sheep. D. VAC bighorn sheep. Sheep in VAC groups were given their first and second vaccine injections the same days samples were collected at weeks 0 and 2, respectively.

group except #7 and #17 which maintained $\log_2$ titers ≥12 and 10, respectively.

Adult domestic sheep in the NV group had relatively stable antibody $\log_2$ titers of 9 to 11 against the T10 antigen (Fig. 3A and 3B). Two <1-yr-old sheep in this group had 4 fold antibody increases during the study period. Similarly, sheep in the VAC group had relatively stable titers with 0 to 4 fold increases. Bighorn sheep in the NV group also had relatively stable titers with 2 fold increases detected for animals #1 and #2 (Fig. 3C and 3D). Similar increases were detected for bighorn sheep in the VAC group with the greatest increase detected in sera from the <1-yr-old bighorn which had an 8 fold increase during the test period.

Leukotoxin neutralizing antibody titers ranged from $\log_2$ 4 to 12 in sera from NV and from $\log_2$ 6 to 12 in VAC domestic sheep (Fig. 4A and 4B). All domestic sheep in the VAC group had 4 to 8 fold increases PV antibody titers in comparison to varied responses over the study period with 0 to 4 fold increases in NV domestic sheep. Neutralizing antibody $\log_2$ titers varied from 0 to 14 in sera from NV bighorns (Fig. 4C). The two NV animals with the highest titers were #18 which had pneumonia as a lamb 4 yr prior to this study and #2 which had pneumonia at 2 yr of age 2 yr prior to this study. Three bighorns, #7, #10, and #17, with high neutralizing titers in the VAC group previously had pneumonia. These three animals had moderate increases in titers in response to vaccination (Fig. 4D). Two others, one <1-yr-old and the other a mature ewe, #9, had 256 fold and 32 fold increases respectively following vaccination. Antibody titers in these two sheep returned to prevaccination levels by 16 wk PV.

Three hundred eleven isolates of *Pasteurella* spp. were cultured from the domestic sheep samples. These isolates were identified in 44 different biovariant groups (data not shown). The largest numbers of organisms were identified in biovariant groups 2 (n = 60) and 1 (n = 55). Simi-

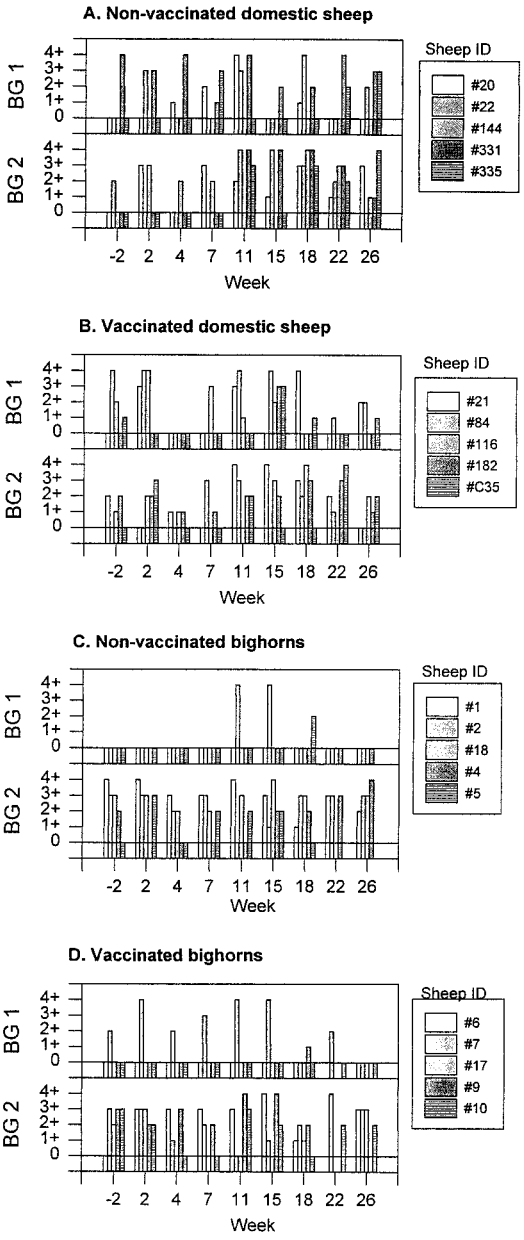

FIGURE 5. Isolation and estimated number of *P. haemolytica* biovariant groups 1 (BG1) and 2 (BG2) colony forming units (CFU) isolated from: A. Non-vaccinated (NV) domestic sheep, B. Vaccinated (VAC) domestic sheep, C. NV bighorn sheep, D. VAC bighorn sheep. Bars below line 0 identify and indicate position for animals that were cultured at each test period. Bars above line 0 indicate the estimated CFU isolated: 0 = no BG1 or BG2 organisms, 1+ = 1–9, 2+ = 10–49, 3+ = 50–100, and 4+ = >100 CFU of BG1 and BG2 isolated from each respective animal.

larly, 342 isolates identified in 45 biovariant groups were cultured from bighorn sheep samples. The largest numbers from bighorn sheep also belonged to biovariant groups 2 (n = 105) and 1 (n = 24). The number of animals from which biogroups 1 and 2 and estimated number of CFU from each animal appeared to vary between groups and over time (Fig. 5). At 4 and 6 wk after the first vaccination 0 of 5 and 1 of 5 domestic sheep in the VAC group were shedding biovariant 1 *Pasteurella* sp. in contrast to 2 of 5 and 3 of 5 sheep in the NV group, respectively (Fig. 5A, B). One NV and two VAC bighorn sheep shed biovariant 1 organisms during the study period. Shedding of biovariant group 2 organisms remained fairly constant in bighorns and appeared to increase in domestic sheep of the NV and VAC groups.

Bighorns #2 in the NV group and #7 in the VAC group developed respiratory disease due to an A2 *P. haemolytica* strain between weeks 15 and 16 PV and were treated with antibiotics. Three days after initiation of treatment of these animals, ewe #5 died during parturition and pen mates appeared to have early signs of respiratory disease with production of increased nasal exudate and coughing. Antibiotic treatment was initiated in the remaining animals but ewe #9 died within 12 hr after treatment was started. *Pasteurella haemolytica* A2 organisms which had the same REA pattern were isolated from nasal and pharyngeal samples of #2 and #7 the uterus of #5 and heart of #9.

DISCUSSION

A primary concern for any product to be used to treat animals, particularly wild animals for which relevant data may be lacking, is safety. The only adverse response to the vaccine used in this study was mild lameness which was detected for only a few days following injection. When the product is a *Pasteurella* vaccine there is also a concern for potential exacerbation of pasteurellosis resulting from immuni-

zation (Bateman, 1988). Although *P. haemolytica* A2 organisms isolated from the heart of one (VAC) and the uterus of the other (NV) bighorn ewe which died is indicative of septicemia, septicemia is a common sequela of pneumonic pasteurellosis in ruminants and disease in these sheep was not indicative of vaccine induced exacerbation. An additional and essential concern in evaluation of a vaccine is its ability to induce protective immunity. Our objectives to evaluate the ability of bighorn sheep to produce humoral antibodies in response to vaccination and to compare those to responses of vaccinated domestic sheep were prerequisite to consideration of experimental challenge of bighorns. Results from this study provides a basis for evaluating antibody titers detected in bighorn and domestic sheep in this study and for considering our findings with those from studies conducted by others in domestic sheep and cattle.

Varying degrees of protection have been achieved in domestic sheep with different pasteurella vaccine preparations (Donachie, 1995). Sutherland et. al. (1988) found that when sheep were immunized with a sodium salicylate extract (SSE) of a medium in which *P. haemolytica* serotype A2 had grown, 47% protection from experimental infection was achieved. Vaccination with a crude leukotoxin preparation resulted in an increase to 86% protection and vaccination with a combination of the two products gave 98% protection. The vaccine used in bighorn sheep in our study contained serotype specific factors as well as leukotoxin and therefore was expected to provide protection comparable to the combined products Sutherland et al. (1988) used in domestic sheep. Jones et al. (1989) determined that disease resistance in domestic sheep correlated with serum antibody titers and that sheep could be passively immunized by intravenous or intraperitoneal infusion of sera from sheep convalescent from experimentally induced pasteurellosis or immunization. Antibodies against antigenic components have been found to confer protection against disease associated with heterologous *Pasteurella* strains as well as the homologous vaccine strains (Donachie, 1995). Mosier et al. (1989) found that vaccination of calves with A1 *P. haemolytica* resulted in production of antibodies against numerous antigens; however, only a portion of the antibodies were found to be associated with disease resistance.

Serum antibodies may also be produced against organisms which colonize mucosal surfaces of the upper respiratory tract. In conventionally reared domestic lambs these surfaces become colonized with *Pasteurella* spp. within hours of birth (Al-Sultan and Aitken, 1985). Although lambs at 4 and 8 wk of age did not have evidence of antibody production against these organisms nor components of a *Pasteurella* vaccine, older conventionally reared domestic sheep develop antibodies, believed to be in response to colonization, and associated resistance to naturally occurring and experimental induced disease (Gilmour and Gilmour, 1989; Donachie, 1995). Antibodies present in sera of sheep in our study prior to vaccination and those in NV groups appear to have resulted, at least in part, due to response to stimulation by organisms which colonized their mucous membranes.

Antibodies reactive against A1 cells were present in sera of all domestic sheep in our study prior to vaccination and adult sheep had much higher titers than sheep <1-yr-old. In contrast to domestic sheep, bighorn sheep >1-yr-old, except those with histories of pneumonia associated with an A1 strain, had antibody titers only slightly higher than bighorn sheep <1-yr-old. The fact that bighorn sheep #2 and #7 had experienced previous A1 infections, produced and retained high antibody titers to that organism, comparable to titers in domestic sheep, and that #6 produced a 32-fold increase of titer in response to vaccination, provides evidence that bighorn sheep are immunologically competent to produce antibodies against A1 organisms.

Pre- and post-vaccination antibody levels to A2 and T10 components were comparable in bighorn and domestic sheep. Most domestic sheep had $\log_2$ titers of 12 to A2 throughout the study period. However, since sera were not diluted beyond this point for A2 and T10 vaccine components, higher titers would not have been detected. Bighorns #2 and #7 had high titers throughout the test period and three additional bighorns in the VAC group had transient rises in antibody titers against A2 cells following vaccination. Although resistance to A2 disease correlates with antibody titers, the capsular polysaccharide associated with A2 specificity is less immunogenic than capsular antigens associated with other serotypes (Adlam, 1989). Protective immunity to an A2 strain was enhanced in domestic sheep by inclusion of iron regulated proteins (IRP) produced by organisms when grown in vivo (Donachie, 1995). Bacteria in the environment besides *P. haemolytica* A2 strains stimulate production of cross-reacting antibodies (Adlam, 1989) and may have been responsible for a portion of the antibodies detected against A2 cells in sera of bighorn and domestic sheep in our study. High antibody titers against T10 cells also were detected in sera from both VAC and NV bighorn and domestic sheep. Biotype T organisms commonly colonize the mucosa of the upper respiratory tract and tonsils of bighorn and domestic sheep. Antibodies detected in the sera of conventionally reared domestic sheep has been attributed to antigenic stimulation from these organisms (Donachie, 1995).

Our data demonstrates that sheep in both species were immunologically competent to produce antibodies against leukotoxin. Serum leukotoxin neutralizing antibodies were consistently high in sera of <1-yr-old and adult domestic sheep in contrast to low titers in both <1-yr-old and adult bighorn sheep without a history of respiratory disease. Leukotoxin neutralizing antibodies resulting from vaccination of bighorns #6 and #9 did not remain elevated in these animals longer than 16 wk PV as it did in bighorns which had experienced pneumonic pasteurellosis or in domestic sheep without a history of disease. Neutralizing antibody titers have also been found to correlate with disease resistance (Gentry et al., 1985, Donachie, 1995). Incorporation of IRP's and leukotoxoid in vaccines may provide a means of stimulating immunity to disease associated with a broad spectrum of biotype A serotypes. Absence of antibodies in serum of NV bighorns without a history of pneumonia may indicate that bighorns without antibodies against this component did not carry strains of *Pasteurella* spp. that produced leukotoxins or that leukotoxins produced by strains normally carried by the bighorns were antigenically distinct from that used in the neutralizing tests.

Vaccination of calves with a *P. haemolytica* A1 vaccine resulted in reduced isolation of A1 strains from nasal and tonsillar samples (Frank et al., 1994). We monitored sheep in our study for similar suppression of A1, A2, and T10 organisms. Biotype A serotype standards 1, 2, 5, 6, 7, 8, 9, 13, and 14 react as strict biogroup 1 organisms and biotype T serotype standards 3, 4, and 10 react as strict biogroup 2 organisms (Jaworski et al., 1998). The fact that biogroup 1 organisms were not isolated from any of the domestic sheep in the VAC group in contrast to isolation from two NV sheep at 4 wk PV is suggestive of some suppression of colonization following vaccination. However, the numbers did not differ enough from those recovered from NV group over the study period to provide conclusive evidence of suppression of colonization by these organisms due to immunity, nor was there any evidence of suppression of biotype T biogroup 2 colonization. Similar results were observed by Miller et al. (1997).

Although experimental challenge was not an objective of this study bighorns #2 and #7 developed respiratory disease after being put back in contact with domestic sheep at 8 wk PV. It was concluded that

disease was transmitted from these animals to #5 and #9 by animal handlers who collected pharyngeal samples from other bighorn sheep after sampling animals #2 and #7. All four animals were treated with antibiotics; #2 and #7 survived in contrast to bighorn ewes #5 (NV) which died without showing prior clinical illness and #9 (VAC) which died less than a day after clinical illness was detected. It appears that the outcome of infections in #2 and #7 were modified by high serum antibody levels.

Experimental challenges of domestic sheep immunized with *Pasteurella* spp. vaccines were conducted within weeks of vaccination and produced less than 100% protection (Sutherland et al., 1988; Jones et al., 1989). Animal #9 which died due to an A2 septicemia 16 wk PV produced 2, 4, and 32 fold antibody increases against A1 and A2 antigens and leukotoxin activity respectively. However, these antibody levels had returned to prevaccination levels by the time of this animal's death, a factor that may have correlated with increased susceptibility to disease. In contrast, bighorn sheep which had prior respiratory disease and domestic sheep without a history of disease sustained high titers over the trial period. Kraabel et al. (1998) found that immunization of bighorn sheep with the same *Pasteurella* A1, A2, and T10 vaccine used in this study reduced mortality in another trial when animals were vaccinated 10 days prior to challenge but failed to provide significant protection for animals vaccinated 57 wk earlier.

It is evident that bighorn sheep were capable of recognizing antigenic components of the *Pasteurella* vaccine and producing antibodies against those components as well as those of organisms with which they were naturally infected. Vaccine induced increases in antibodies against the vaccine components were very transient in comparison to those induced by natural infection. Protective antibodies should be maintained at high levels over a period of years for optimum protection of bighorn sheep from disease associated with *Pasteurella.* spp. A *Pasteurella* vaccine for use in bighorn sheep maintained in captivity and at times of capture for relocation could help to reduce mortalities associated with pasteurellosis. Such a vaccine should be designed for ease of administration, enhancement of antigenicity to stimulate and maintain high levels of antibodies, and should contain antigenic complexes which would stimulate production of protective antibodies that would cross react with and provide immunity against disease associated with multiple strains of *Pasteurella.*

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