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PASTEURELLA SPP. IN SYMPATRIC BIGHORN AND DOMESTIC SHEEP

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ABSTRACT: Domestic sheep were sighted at different times from 1991 to 1993 on four Nevada (USA) ranges occupied by bighorn sheep. Nasal and pharyngeal swab samples were collected from both sheep species and cultured to determine if any strains of *Pasteurella* spp. were shared on range conditions after contact of the two species. *Pasteurella* spp. were isolated from all 38 bighorn sheep and 16 of 17 domestic sheep included in this study. The isolates were characterized on the bases of species, biotype, serotype, biogroup, and restriction enzyme analyses (REA) as well as ribotyping of bacterial DNA. A *P. haemolytica* biotype 3, biogroup 11 isolate from a domestic sheep had biochemical, REA, and ribotype profiles which were identical to those of isolates from three bighorn sheep on the same range. None of the other isolates were found to be common to the two sheep species. Disease was not detected in any of the bighorn populations. However, bighorn sheep populations were extirpated on two ranges while increasing on the other two, including the range on which *P. haemolytica* biotype 3, biogroup 11 strain was isolated. Declining sheep numbers were not correlated with the presence of any one strain of *Pasteurella* spp from the sheep.

Key words: *Pasteurella* spp., biogroups, restriction enzyme analysis, bighorn sheep, (*Ovis canadensis*), domestic sheep (*Ovis aries*), epizootiology.

INTRODUCTION

Interaction of domestic sheep (*Ovis aries*) and bighorn sheep (*O. canadensis*) has the potential for transmission of infectious agents associated with disease. *Pasteurella* spp. are the most frequently identified infectious agents associated with respiratory disease in both sheep species. Therefore, when domestic sheep were observed in bighorn habitat we collaborated to determine if interaction resulted in colonization of both sheep species with identical strains of *Pasteurella* sp. and if there was evidence of subsequent deleterious affects in bighorn populations.

Pasteurella spp. are part of the normal flora of the nasal mucosa and tonsillar crypts of both bighorn and domestic sheep (Biberstein et al., 1970; Al-Sultan and Aitken, 1985; Ward et al., 1990; Queen et al., 1994). Naturally occurring respiratory disease, due to *Pasteurella* spp. has been documented in both animal species (Gilmour, 1980; Onderka and Wishart, 1984; Ward et al., 1992). However, in some instances, deaths of bighorn sheep have been attributed to infection with *Pasteurella* spp. transmitted from domestic sheep (Foreyt and Jessup, 1982; Onderka and Wishart, 1988). Although conclusive evidence of transmission is lacking in these cases, strains of *Pasteurella* spp carried by domestic sheep may be more pathogenic for bighorn than for domestic sheep and may cause long term health problems following introduction (Foreyt, 1990).

To determine if organisms carried by one animal are the same as those carried by another requires characterization of both phenotypic and genetic traits. Traditionally *Pasteurella* spp. isolates are characterized by biochemical tests to identify species and biotypes (Kilian and Frederiksen, 1981). The genus was divided in the latest edition of Bergey's Manual of Systematic Bacteriology into six species; *aerogenes*, *gallinarum*, *haemolytica*, *multocida*, *pneumotropica*, and *ureae* based on biochemical reactions (Mannheim, 1984).

Pasteurella haemolytica was separated into three biotypes (A, T, and 3) (Kilian and Frederiksen, 1981). However, additional species designations have subsequently been created; resulting in a number of recommendations for name changes within the genus (Holt et al., 1994) including a change in the name of *P. haemolytica* biotype T organisms to *P. trehalosi* (Sneath and Stevens, 1990). Members of this group are common in sheep species and make up a major portion of the organisms cultured in this study, therefore; we will use the newer designation, *P. trehalosi*, in this report along with the more familiar "biotype T" designation for clarity.

The *P. haemolytica*, including biotype A, 3, and T (*P. trehalosi*), and *P. multocida* groups are most commonly associated with disease in ruminants. Members of these species have been divided further into serotypes based on detection of specific bacterial antigens (Carter, 1955; Biberstein et al., 1960; Heddleston et al., 1972; Frank and Wessman, 1978). Serotypes identified in the catalase-positive *P. haemolytica* biotypes A and 3 include 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, and 16, while catalase-negative biotype T (*P. trehalosi*) isolates include serotypes 3, 4, 10 and 15. In our experience, serotyping of *P. haemolytica* isolates is problematic since a large percentage of biotypes A and 3 isolates from wild ruminants autoagglutinate or do not agglutinate in any of the available typing sera and most biotype T (*P. trehalosi*) isolates agglutinate in multiple antisera.

A combination of the above procedures are not sensitive or reliable enough to determine if specific strains of *Pasteurella* spp. are present in more than one animal or group of animals. Therefore, we added procedures with greater discriminatory capabilities. Biochemical tests used to divide isolates into additional biotypes (Biberstein et al., 1991) and biogroups (Bisgaard and Mutters, 1986), and DNA fingerprinting by restriction enzyme analysis (REA) and ribotyping (Jaworski et al., 1993) were coupled for this task. These techniques reliably demonstrated transmission between bighorn ewes and their caesarian-derived lambs (Jaworski et al., 1993) and between domestic and bighorn sheep allowed fence-line contact (D. L. Hunter and A. C. S. Ward, unpubl. data).

This study includes data from samples collected December 1991 through December 1993. Bighorn sheep populations on four ranges and isolates of *Pasteurella* spp from these sheep and domestic sheep removed from the ranges were evaluated. In addition bighorn population data was evaluated from prior to and following contact with domestic sheep in an attempt to determine if bighorn populations were deleteriously affected.

MATERIALS AND METHODS

Domestic sheep (*Ovis aries*) were sighted on four bighorn (*Ovis canadensis*) range sites: East Range (40°12′ to 40°16′N, 117°50′ to 117°54′W), Tobin Mountain (40°21′ to 40°26′N, 117°29′ to 117°33′W), Desatoya Mountains (39°18′ to 39°22′N, 117°49′ to 117°53′W), and the Granite Range (40°47′ to 41°50′N, 119°25′ to 119°27′W) in Nevada, USA.

Domestic sheep included four adult females captured from the East Range within an estimated 400 m of the main water source used by bighorn sheep, an adult female and her lamb from the Desatoya Range and a single castrated male approximately 6-mo old from the Granite Range. In addition, 10 domestic sheep from a flock on a ranch adjacent to the Tobin Range were tested. A portion of this flock grazed on the Tobin Range during the preceding summer. The length of time that the domestic sheep were on the various ranges was not known. It is estimated that domestic sheep trespassed on the Tobin Range for 2 to 4 wk during the 1991 grazing season. Interaction of domestic sheep and bighorn sheep on the Tobin Range was probable but the duration of contact is unknown. Those on the East Range were removed approximately 2 wk after they were first observed in close proximity to the water sources for bighorns in the fall of 1991. Interactions of bighorn sheep with domestic sheep was very probable since they would have shared a common water source. The domestic ewe and her undocked lamb were observed with bighorns prior to their removal from the Desatoya Range in the fall of 1992. A domestic castrated male sighted with bighorn sheep on

the Granite Range was judged to have been there for approximately 2 wk prior to removal in October of 1992.

Bighorn sheep captured for testing included one on the Tobin Range in January 1992; seven (four in January 1992 and three in January 1993) on the East Range; 15 sheep on the Desatoyas with six, five, and four captured in January, May, and December 1993, respectively; and 15 sheep on the Granite Range with four captured in October 1992, and two, five, and four captured in January, May, and December of 1993, respectively. A numbered ear tag was placed in an ear of each captured animal. None of the animals were recaptured for subsequent sampling.

All bighorn sheep tested and domestic sheep on the Desatoya and Granite Ranges were captured by net-gunning (Kock et al., 1987). Nets were deployed by a gunner working from a helicopter. Domestic sheep which trespassed on the Tobin Range were herded from the range and those taken from the East Range were caught by hand and placed in a stock trailer for transport to holding pens in Winnemucca, Nevada.

Nasal and pharyngeal swab samples were collected from each captured animal. Nasal samples were collected using rayon tipped swabs supplied with Amies transport medium with charcoal (Precision Dynamics Corporation, San Fernando, California, USA). Swab samples of the pharyngeal area were collected using Accu-CulShure® (Accu-Med Corporation, Pleasantville, New York, USA) collection and transport systems.

Samples were inoculated onto bacterial culture media within 12 hr of collection. Culture media included Columbia blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) with 5% ovine blood (CBA), and a Columbia blood agar with 5% bovine blood agar plus antibiotics (CBAA) selective for Pasteurellaceae (Ward et al., 1986). Inoculated media were placed directly in a 35 C incubator with 10% added CO_2, or in a styrofoam box with a thermometer and bottles filled with hot tap water maintained between 22 and 38 C while additional captures were conducted. Upon return to the Caine Veterinary Teaching and Research Center (CVTRC) the inoculated media were placed in a CO_2 incubator maintained at 35 C. Cultures were examined after 24 and 48 hr incubation and evaluated for growth of colonies resembling *Pasteurella* spp. A representative of each type of *Pasteurella* spp.-like colony, which varied by size, color, appearance of surface texture and hemolytic activity, was selected for identification. Two to ten colony types were evaluated from cultures of each animal using the techniques of Kilian and Frederiksen (1981).

Gram-negative isolates which were oxidase-positive and did not grow or grew poorly on MacConkey's agar (Becton Dickinson Microbiology Systems) had the basic reactions characteristic of *Pasteurella* spp. Such isolates were biogrouped using discriminatory tests from the procedure of Bisgaard and Mutters (1986). Additional substrates were used to test indole-positive isolates (Biberstein et al., 1991). Slide agglutination tests were conducted on biotypes A (*P. haemolytica*) and T (*P. trehalosi*) isolates for serotype identification (Frank and Wessman, 1978). Biotype 3 and indole-positive *Pasteurella* spp. were not serotyped.

Biochemically identical isolates cultured from both domestic and bighorn sheep within a geographic area were subjected to restriction enzyme analysis (REA) and ribotyping to determine if any strains were common to the two sheep species. Bacterial DNA was harvested for REA from growth propagated from a single colony of each isolate using the procedures of Jaworski et al., (1993). Restriction enzyme *Hae* III (International Biotechnology, Inc. New Haven, Connecticut, USA) was used to digest DNA preparations prior to gel electrophoresis in 0.6% LE agarose (FMC BioProducts, Rockland, Maine, USA). Selected isolates were also digested with *Hpa* II and *Hha* I restriction enzymes (International Biotechnology, Inc.). Gels subsequently were stained with 0.05% ethidium bromide (International Biotechnology, Inc.) and photographed with a red filter while exposed to UV light to record profiles of cut DNA. The DNA was then transferred to nylon membranes (Boehringer Mannheim, Indianapolis, Indiana, USA) and hybridized with a digoxigenin-labelled ribosomal RNA (Boehringer Mannheim) probe for ribotyping (Jaworski et al., 1993). Biotype T (*P. trehalosi*) isolates representative of REA types were selected for parallel testing in a single gel for demonstration of variances. Similarity coefficients (S_{AB}s) were calculated for bands in photographs of similar REA profiles in a single gel (Schmid et al., 1990).

Data on the bighorn population dynamics for each of the four habitats from 1989 through 1995 were obtained from the Nevada Division of Wildlife population records.

RESULTS

Pasteurella spp. were isolated from all bighorn and all but one domestic sheep. Data for isolates from nasal and pharyngeal samples were combined for each an-

TABLE 1. *Pasteurella* isolated from bighorn (EBH) and domestic sheep (EDS) with potential contacts on the Nevada East Range.

Animal number	*Pasteurella* isolated[a]	Biotype	Serotype[b]	Biogroup[c]	REA types[d]	Ribotype[d]	Date mo/year
EDS-1	P. haem	A	2	1	ND	ND	12/91
	P. treh	T	3, 4, 10	2	NT-10	NRT-T-10	12/91
	P. canis A	NA[e]	ND	NA[e]	NI+1	NRT-I-101	12/91
EDS-2	P. haem	A	UT	1	ND	ND	12/91
	P. treh	T	10	4CD	NT-11	ND	12/91
EDS-3	P. haem	A	2	1	ND	ND	12/91
	P. haem	A	8	UBEL	ND	ND	12/91
	P. treh	T	10	2	NT-12	NRT-T-12	12/91
EDS-4	P. haem	A	UT	1	ND	ND	12/91
	P. treh	T	3, 10	4CD	NT-13	ND	12/91
EBH-1	P. haem	3	ND	3B	ND	ND	1/92
	P. treh	T	10	2	NT-1	ND	1/92
	P. canis A	NA	ND	NA	NI+2	NRT-I-103	1/92
EBH-2	P. haem	A	UT	UAL	ND	ND	1/92
	P. treh	T	3	2	NT-4	NRT-T-2	1/92
	P. canis A	NA	ND	NA	NI+2	NRT-I-103	1/92
EBH-3	P. treh	T	3, 4, 10	2	NT-2	ND	1/92
	P. canis A	NA	ND	NA	NI+2	ND	1/92
EBH-3329	P. haem	3	ND	3	ND	ND	1/92
	P. treh	T	3, 4	2	NT-2	NRT-T-1	1/92
	P. treh	T	3, 4, 10	2CD	NT-3	ND	1/92
EBH-21	P. treh	T	3, 4, 10	2	NT-1	NRT-T-1	1/93
	P. haem	3	ND	10B	N3-3	NRT-3-2	1/93
EBH-22	P. treh	T	3, 4, 10	2	NT-1	ND	1/93
	P. haem	3	ND	10B	N3-3	NRT-3-2	1/93
EBH-23	P. treh	T	3, 4, 10	2	NT-1	ND	1/93
	P. haem	3	ND	10B	N3-2	NRT-3-3	1/93

[a] P. haem. = *P. haemolytica*, P. treh = *P. trehalosi*, and *P. canis* A (an indole positive isolate).

[b] Biotypes A and T were serotyped, UT = untypable with available antisera, ND = not done for biotype 3 and indole positive isolates.

[c] The letter "U" or a number alone indicates strict biogroup designation, a U or a number followed by additional letters indicates an isolate which biochemically varies from those identified by Bisgaard and Mutter (1986).

[d] Restriction enzyme analysis (REA) and ribotype (RT) patterns: ND = not done, with others the first letter, N = Nevada, with the biotype designations; A, 3, T, for *P. haemolytica/trehalosi* biotypes and I for indole producing species and biotypes.

[e] NA = not applicable, used for isolates which are identified by species but not biotype or biogroup such as *P. canis* A.

imal. Only one representative for each isolate type per individual animal is presented (Tables 1, 2, 3, and 4). We evaluated 120 isolates, including 51 biotype T, 34 biotype A, 22 biotype 3, and 13 indole-positive isolates. Biotype T (*P. trehalosi*) isolates were cultured from 35 (92%) of the 38 bighorn sheep but only six (35%) of 17 domestic sheep. These organisms were cultured from both domestic and bighorn sheep on the East, Tobin and Desatoya Ranges, but not from the domestic sheep removed from the Granite Range. In contrast, biotype A isolates were cultured from 14 (82%) of domestic sheep but only 13 (34%) of bighorns tested. These organisms were isolated from bighorns on the East and Desatoya Ranges but not from bighorns captured on the Tobin or Granite Ranges. Biotype 3 *P. haemolytica* were isolated from four (24%) of the domestic and 18 (47%) of the bighorn sheep. Biotype 3 organisms were isolated from both domestic and bighorn sheep on the Desatoya and Granite Ranges and from the bighorn sheep on and domestic sheep associated

TABLE 2. *Pasteurella* isolated from bighorn (DBH) and domestic sheep (DDS) with potential contacts on the Desatoya Mountain Range in Nevada.

Animal number	*Pasteurella* isolated[a]	Biotype	Serotype[b]	Biogroup[c]	REA types[d]	Ribotype[d]	Date mo/year
DDS-1	P. haem	A	2	1	NA-1	NRT-A-1	11/92
	P. haem	A	2	1A	ND	ND	11/92
	P. haem	3	ND	5	ND	ND	11/92
	P. haem	A	2	16E	NA-5	ND	11/92
	P. treh	T	3	2	NT-20	NRT-T-20	11/92
DDS-2	P. haem	A	2	1	NA-1	NRT-A-1	11/92
	P. haem	A	2	1A	ND	ND	11/92
	P. haem	3	ND	5	ND	ND	11/92
	P. haem	A	UT	UBLX	NA-6	ND	11/92
	P. haem	A	UT	7X	NA-7	ND	11/92
DBH-1	P. haem	A	2	1	NA-2	NRT-A-2	1/93
	P. treh	T	3, 4	2	NT-21	NRT-T-21	1/93
DBH-2	P. treh	T	3, 4, 10	2	UR[e]	NRT-T-24	1/93
	P. treh	T	3, 10	2	NT-22	NRT-T-22	1/93
DBH-3	P. haem	A	UT	1BL	NA-3	ND	1/93
	P. haem	3	UT	11A	ND	ND	1/93
	P. treh	T	3, 4	2	NT-23	NRT-T-23	1/93
DBH-4	P. haem	A	UT	1B	NA-4	ND	1/93
	P. treh	T	3, 4	2	NT-24	NRT-T-22	1/93
DBH-5	P. haem	A	UT	1B	NA-4	ND	1/93
	P. treh	T	3, 4, 10	2	NT-25	NRT-T-25	1/93
	P. treh	T	4	2	NT-26	NRT-T-25	1/93
DBH-6	P. treh	T	3, 4, 10	2	NT-27	NRT-T-22	1/93
	P. treh	T	UT	2	UR	NRT-T-24	1/93
DBH-7	P. haem	A	8	1AB	ND	ND	5/93
	P. treh	T	3, 4, 10	2	NT-24	ND	5/93
DBH-8	P. treh	T	3, 4, 10	2	NT-24	ND	5/93
DBH-9	P. haem	3	ND	3	ND	ND	5/93
DBH-10	P. haem	A	UT	1B	NA-4	ND	5/93
	P. haem	T	3, 4, 10	2	NT-21	ND	5/93
DBH-11	P. haem	T	3, 4	2	NT-21	ND	5/93
DBH-12	P. haem	A	UT	1B	NA-4	ND	12/93
DBH-13	P. haem	T	4	2	NT-24	ND	12/93
DBH-14	P. haem	A	UT	U	ND	ND	12/93
	P. treh	T	UT	2	UR	ND	12/93
DBH-15	P. haem	A	UT	1B	NA-4	ND	12/93

[a] P. haem = *P. haemolytica*, P. treh = *P. trehalosi*.

[b] Biotypes A and T were serotyped, UT = untypable with available antisera, ND = not done for biotype 3.

[c] The letter "U" or a number alone indicates strict biogroup designation, a U or a number followed by additional letters indicates an isolate which biochemically varies from those identified by Bisgaard and Mutter (1986).

[d] Restriction enzyme analysis (REA) and ribotype (RT) patterns from *Hae* III cut DNA; ND = not done, with others the first letter, N = Nevada, with the biotype designations; A, 3, T, for *P. haemolytica/trehalosi* biotypes.

[e] UR = Unresolved banding by REA due to incomplete cutting with restriction enzyme. Isolates differentiated by ribotypes.

with the Tobin Range. Indole-producing *Pasteurella* spp. were isolated from four (24%) of domestic and nine (24%) of bighorn sheep. They were isolated from both species on the East Range, from domestic sheep associated with the Tobin Range, and from five (33%) of 15 bighorn sheep tested on the Granite Range but not from either sheep species on the Desatoya Range. Indole-positive organisms included

TABLE 3. *Pasteurella* isolated from bighorn (TBH) and domestic sheep (TDS) with potential contacts on the Tobin Range.

Animal number	*Pasteurella* isolated[a]	Biotype	Serotype[b]	Biogroup[c]	REA types[d]	Ribotype[d]	Date mo/year
TBH-1	P. haem	3	ND	10B	N3-4	NRT-3-4	1/92
	P. treh	T	UT	2S	ND	ND	1/92
TDS-1	P. haem	A	7	1	ND	ND	1/92
	Indole +	U18	NA[e]	NA	NI+3	NRT-I-100	1/92
TDS-2	Negative for Pasteurella						
TDS-3	P. haem	A	6	1	ND	ND	1/92
TDS-4	P. haem	A	2	1	ND	ND	1/92
TDS-5	P. haem	A	2	1	ND	ND	1/92
	P. canis A	NA	ND	NA	NI+1	NRT-I-101	1/92
TDS-6	P. haem	A	2	1	ND	ND	1/92
	P. haem	A	8	1	ND	ND	1/92
	P. haem	A	UT	16E	NA-8	ND	1/92
	P. treh	T	3, 4, 10	2CDE	ND	ND	1/92
TDS-7	P. haem	3	ND	10	N3-5	ND	1/92
TDS-8	P. haem	A	2	1	ND	ND	1/92
	P. haem	A	6	1	ND	ND	1/92
TDS-9	P. haem	A	UT	1	ND	ND	1/92
TDS-10	P. haem	A	UT	1	ND	ND	1/92
	Indole +	U18	ND	NA	NI+4	NRT-I-100	1/92

[a] P. haem = *P. haemolytica*, P. treh = *P. trehalosi*, P. canis A and Indole+ = indole positive isolates.

[b] Biotypes A and T were serotyped, UT = untypable with available antisera, ND = not done for biotype 3 and indole positive isolates.

[c] The letter "U" or a number alone indicates strict biogroup designation, a U or a number followed by additional letters indicates an isolate which biochemically varies from those identified by Bisgaard and Mutter (1986).

[d] Restriction enzyme analysis (REA) and ribotype (RT) patterns: ND = not done, with others the first letter, N = Nevada, with the biotype designations; A, 3, T, for *P. haemolytica/trehalosi* biotypes and I for indole producing species and biotypes.

[e] NA = not applicable, used for isolates which are identified by species but not biotype or biogroup such as *P. canis* A.

P. canis A, which were isolated from one domestic and three bighorn sheep on the East Range, and biotype U18 isolated from two domestic sheep on the ranch adjoining the Tobin Range. Additional indole-positive isolates were identified as *Pasteurella multocida* subspecies *multocida* b and biotypes U16 and U23 which were isolated from bighorn sheep on the Granite Range.

Serotyping results for biotype T (*P. trehalosi*) and *P. haemolytica* biotype A isolates are presented in (Tables 1, 2, 3, and 4). Although biotype 3 isolates were not serotyped, most domestic sheep isolates were differentiated from those of bighorns by biogrouping. Many (19 of 51) biotype T (*P. trehalosi*) isolates agglutinated in antisera for serotypes 3, 4, and 10. However, some agglutinated in only one of the three antisera and 13 of the isolates did not agglutinate in any of the antisera. Of the 34 biotype A isolates, 15 (7 from domestic and 8 from bighorn sheep) were untypable with antisera for serotypes 1, 2, 5, 6, 7, 8, 9, 11, and 12. Thirteen isolates from domestic sheep removed from East, Granite, and Desatoya Ranges, and domestic sheep associated with the Tobin Range, and from a bighorn on the Desatoya Range, were typed as serotype 2. The remaining biotype A isolates included serotypes 6 and 7 organisms from domestic sheep and serotype 8 organisms isolated form both domestic and bighorn sheep.

Biogrouping was conducted on all isolates. Biotype T (*P. trehalosi*) isolates were identified as biogroup 2 or variants of biogroups 2 and 4 (Tables 1, 2, 3, and 4). Biogroup 2 and biogroup 2 variants were iso-

TABLE 4. *Pasteurella* isolated from bighorn (GBH) and domestic sheep (GDS) with potential contacts on the Granite Mountian Range.

Animal number	*Pasteurella* isolated[a]	Biotype	Serotype[b]	Biogroup[c]	REA types[d]	Ribotype[d]	Date mo/year
GBH-1	P. haem	3	ND	3AB	ND	ND	10/92
	P. haem	3	ND	3B	ND	ND	10/92
	P. treh	T	UT	2	ND	ND	10/92
	P. treh	T	4	2	ND	ND	10/92
GBH-2	P. haem	3	ND	6	ND	ND	10/92
	P. treh	T	UT	2	ND	ND	10/92
	P. treh	T	UT	2CD	ND	ND	10/92
GBH-3	P. haem	3	ND	6	ND	ND	10/92
	P. treh	T	3, 4, 10	2	ND	ND	10/92
	P. mult b	NA[e]	ND	NA	NI+5	NRT-I-104	10/92
GBH-4	P. haem	3	ND	6	ND	ND	10/92
	P. treh	T	3, 4, 10	2CD	ND	ND	10/92
GDS-1	P. haem	A	2	1	ND	ND	10/92
	P. haem	3	ND	11	N3-1	NRT-3-1	10/92
GBH-5	P. treh	T	UT	2	ND	ND	1/93
	P. treh	T	UT	2CD	ND	ND	1/93
GBH-6	P. haem	3	ND	11	N3-1	NRT-3-1	1/93
	P. treh	T	3, 4, 10	2	ND	ND	1/93
	P. treh	T	UT	2	ND	ND	1/93
GBH-7	P. haem	3	ND	6	ND	ND	5/93
	P. treh	T	UT	2	ND	ND	5/93
GBH-8	P. treh	T	3, 4	2CD	ND	ND	5/93
	Indole +	NA	ND	U16	NI+6	ND	5/93
	Indole +	NA	ND	U23	ND	ND	5/93
GBH-9	P. haem	3	ND	11	N3-1	ND	5/93
	P. treh	T	3, 4, 10	2	ND	ND	5/93
GBH-10	P. treh	T	3, 4, 10	2	ND	ND	5/93
	P. treh	T	UT	2	ND	ND	5/93
GBH-11	P. treh	T	3, 4	2	ND	ND	5/93
GBH-12	P. haem	3	ND	11	N3-1	NRT-3-1	12/93
	P. treh	T	3, 10	2	ND	ND	12/93
	Indole +	U16	ND	NA	NI+6	ND	12/93
GBH-13	P. treh	T	10	2	ND	ND	12/93
	P. treh	T	UT	2	ND	ND	12/93
	P. mult b	NA	ND	NA	ND	ND	12/93
GBH-14	P. treh	T	3, 4, 10	2	ND	ND	12/93
	Indole +	U16	ND	NA	NI+6	ND	12/93
GBH-15	P. haem	3	ND	3BCE	ND	ND	12/93
	P. treh	T	UT	2	ND	ND	12/93

[a] P. haem = *P. haemolytica*, P. treh = *P. trehalosi*, P. mult b = *P. multocida* b and indole producing isolates.

[b] Biotypes A and T isolates were serotyped, UT = untypable with available antisera, ND = not done for biotype 3, *P. multocida*, or indole positive isolates.

[c] The letter "U" or a number alone indicates strict biogroup designation, a U or a number followed by additional letters indicates an isolate which biochemically varies from those identified by Bisgaard and Mutter (1986).

[d] Restriction enzyme analysis (REA) and ribotype (RT) patterns: ND = not done, with others the first letter, N = Nevada, with the biotype designations; A, 3, T, for *P. haemolytica/trehalosi* biotypes and I for indole producing species and biotypes.

[e] NA = not applicable, used for isolates which are identified by species but not biotype or biogroup such as *P. multocida* b or other indole positive isolates.

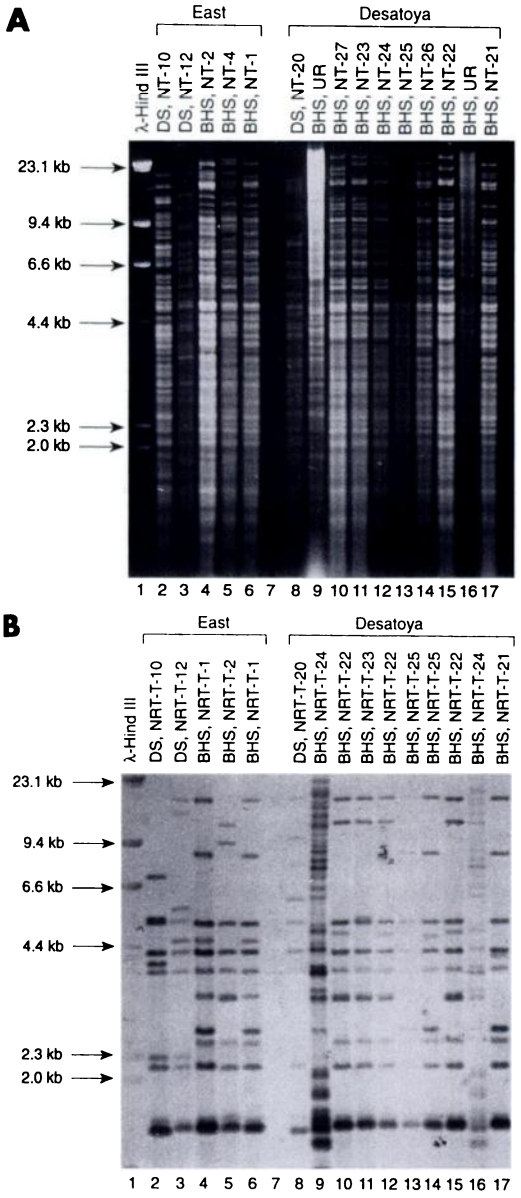

FIGURE 1. Comparison of electrophoresis profiles of restriction enzyme *Hae* III cut DNA from biotype T, biogroup 2 (*P. trehalosi*) isolates from domestic sheep (DS) in lanes 2, 3, and 8 and bighorn sheep (BHS) in lanes 4 through 6 and 9 through 17. a). Ethidium bromide stained 0.6% LE agarose gel photographed with UV illumination. All profiles of bighorn sheep isolates are different from those of domestic sheep isolates. Profiles in lanes 9 and 16 were poorly defined by UV illumination but have identical ribotype profiles. b). DNA was transferred from the above gel to a nylon membrane and hybridized with digoxigenin-labelled ribosomal RNA to produce ribotype profiles. Variances between DNA from bighorn and domestic sheep isolates are evident. Profiles

lated from bighorn sheep on the Tobin, East, and Desatoya Ranges and from domestic sheep removed from or associated with those Ranges. Biogroup 4 variants were isolated from domestic sheep from the East Range. Strict biogroup 1 isolates were isolated from domestic sheep associated with the East, Tobin and Granite Ranges. Biotype A, strict biogroup 1 and biogroup 1 variants, were isolated from both domestic and bighorn sheep on the Desatoya Range. Biotype 3, biogroup 11 isolates, were cultured from three bighorn sheep and a domestic sheep on the Granite Range (Table 4). This organism was isolated from the domestic sheep at the time it was removed from the Granite Range in October 1992, from two bighorns in January 1993, and from one bighorn sheep in November 1993.

The DNA from isolates biochemically identical and cultured from both domestic and bighorn sheep within a geographic area were evaluated by REA and ribotyping. Using these procedures to evaluate biotype T (*P. trehalosi*) biogroup 2 isolates resulted in differentiation of 13 REA types and 10 ribotypes (Figs. 1a and 1b). Additional REA types were detected for biogroup 2 and 4 variants. Three different REA types (NT-22, NT-24, and NT-27) shared a common ribotype (NRT-T-22); REA types NT-25 and NT-26 both had shared ribotype NRT-T-25; and two others (NT-1 and NT-2) shared the ribotype NRT-T-1 profile (Tables 1, 2, 3 and 4). The DNA from two bighorn sheep isolates (Fig. 1a, lanes 9 and 16) did not cut satisfactorily with the *Hae* III restriction enzyme to yield distinct bands with ethidium bromide. Ribotype profiles of the bighorn isolates were similar with each other (Fig. 1b, lanes 9 and 16) but distinct from those of domestic sheep isolates (Fig. 1b, lanes

←

in lanes 9 and 16 appear identical. Kilobase (kb) sizes for λ *Hind* III restriction enzyme fragments in lane 1 are listed in the left-hand margin.

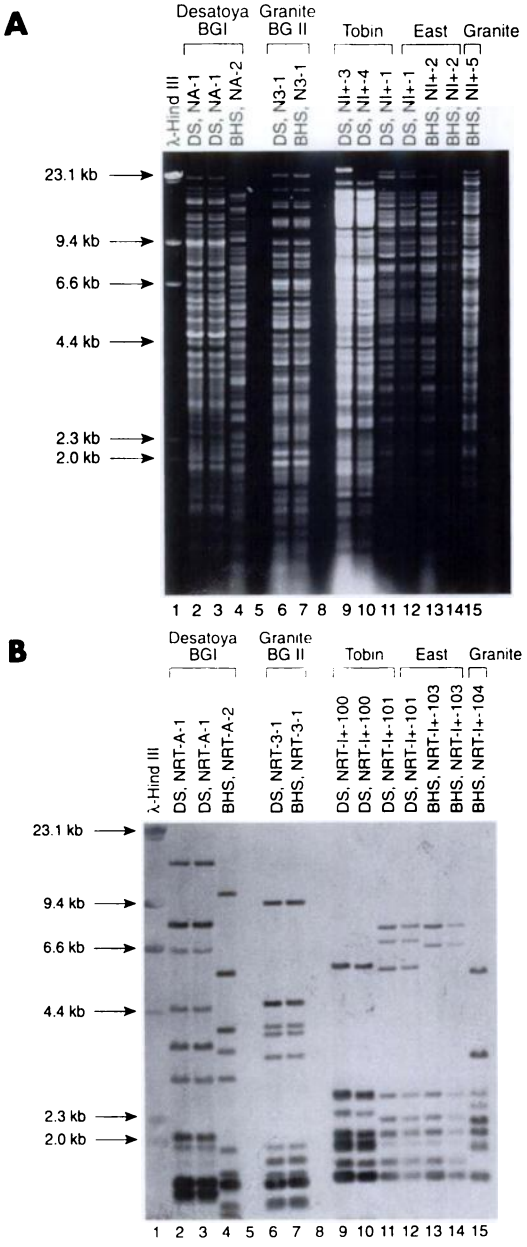

FIGURE 2. Comparisons of electrophoresis profiles of restriction enzyme *Hae* III cut DNA from *Pasteurella haemolytica* biotype A (lanes 2 through 4) and biotype 3 (lanes 6 and 7) and indole producing *Pasteurella* isolates (lanes 9 through 15 for domestic (DS) and bighorn (BHS) sheep. a). Ethidium bromide stained 0.6% LE agarose gel photographed with UV illumination. All profiles of bighorn sheep isolates are different from those of domestic sheep isolates. Profiles of indole producing isolates from domestic sheep associated with the Tobin (lane 11) and East (12) Ranges appear identical. b). Ribotype profiles of the above strains are demonstrated. Differences between BHS and DS isolates are clearly evident. The third band from the top in lane 11 appears to be slightly lower than that in lane 12, which might indicate slight differences between the two DS isolates. Kilobase (kb) sizes for λ *Hind* III restriction enzyme fragments in lane 1 are listed in the left-hand margin.

2, 3, and 8). Therefore, none of the biotype T strains subjected to REA were shared by bighorn and domestic sheep.

The REA and ribotype profiles of *P. haemolytica* A2 biogroup 1 isolates cultured from the two domestic sheep removed from the Desatoya Range were identical with each other but distinct from that of the single A2 isolate from a bighorn sheep (Figs. 2a and 2b; lanes 2 to 4). However, the REA and ribotype profiles of the four biotype 3, biogroup 11 isolates from sheep on the Granite Range, two of which are included in Figs. 2a and 2b (lanes 6 and 7), appear to be identical (S_{AB} = 1.0).

Ribotype and REA profiles of indole-positive *Pasteurella* spp. varied for domestic and bighorn isolates (Figs. 2a and 2b; lanes 9 to 15), with none being shared by the two sheep species. An isolate from a domestic sheep in the herd which trespassed on the Tobin Range had a REA pattern which appeared identical to that of an isolate from a domestic sheep on the East Range (Figs. 2a and 2b, lanes 11 and 12) when DNA was cut with *Hae* III (S_{AB} 1.0). However, minor differences of fragments at approximately 4.6 kilobases (kb) were noted in ribotype profiles of the two isolates. When DNA preparations from the two isolates were cut with *Hpa* II and *Hha* I, REA patterns were calculated to have S_{AB} values of 0.96 and 0.93, respectively (Figs. 3a and 3b). These were the only organisms isolated from animals on more than one range which had S_{AB} values near 1.0.

Several biotype T (*P. trehalosi*) biogroup 2 strains persisted over a period of months on the East and Desatoya Ranges (Tables 1 and 2). Biogroup 2, NT-1 isolates were cultured from bighorn sheep on the

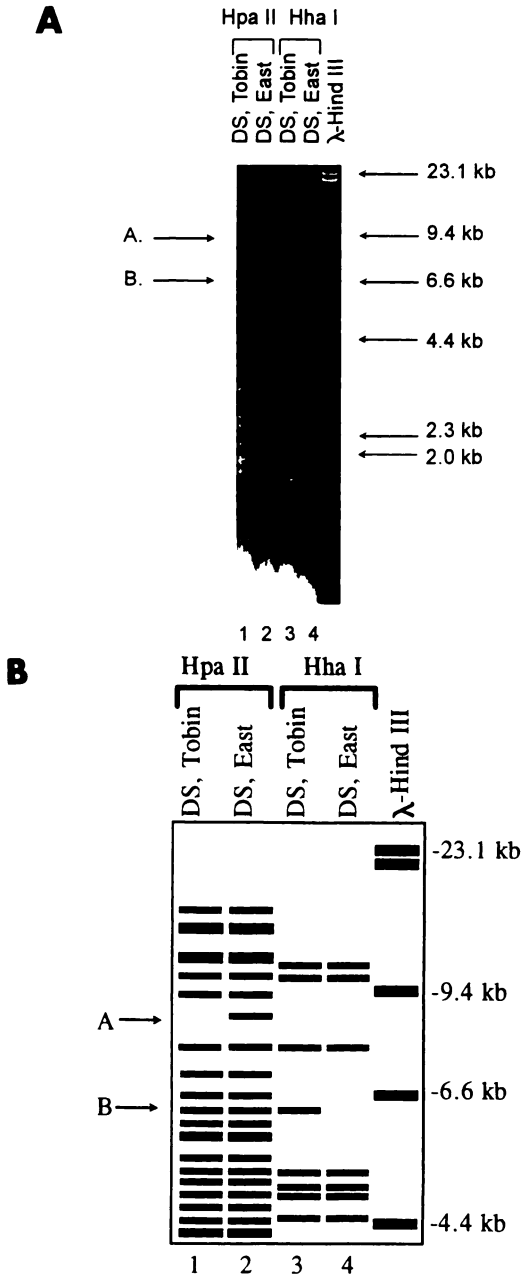

FIGURE 3. Comparison of NI + 1 isolates from domestic sheep from Tobin and East Ranges cut with *Hpa* II and *Hha* I restriction enzymes (Fig. 3a). The image clarity of DNA fragments in photographs of the profiles was lost in the process of transfer for preparation of figures. Therefore, enhancement of profile variances was produced (Fig. 3b). a) A band of approximately 9.1 kb present in the *Hpa* II cut DNA of the isolate from the sheep removed from the East Range is not detectable in the DNA fragments of the isolate from the sheep associated with the Tobin range (see generated enhanced image in b). The calculated S_{AB} for these DNA profiles was 0.96. Similarly, when the DNA from each isolate was cut with *Hha* I, a band of approximately 6.5 kd was evident in the lane 3 but not in the DNA fragments in lane 4. (see generated enhanced image in b). Other bands which were present in both profiles varied in intensity and the calculated S_{AB} value was 0.93. Kilobase (kb) sizes for λ *Hind* III restriction fragments in lane 1 are listed in the right-hand margin.

East Range in January 1992 and January 1993 and a biogroup 2, NT-24 strain was isolated from Desatoya bighorns in January and May 1993. In addition, a biotype A, NA-4 strain was recovered from Desatoya bighorns in January and May 1993.

The bighorn population on the East Range was established in 1984 when 24 sheep were moved to that location from the River Mountain area in Southern Nevada. The numbers of bighorns on the East Range were determined by aerial surveys conducted in 1989, 1990, and 1991 to be 36, 31, and 22 respectively. The number of sheep observed in 1992 was further reduced, nine bighorns were sighted in March of 1993 and no sheep were sighted on this range in 1994 or 1995. Forty-five bighorns were also moved from the River Mountain area in 1984 and released on the Tobin Range. The population was augmented with 18 more bighorns from the Lake Mead population in October of 1991. Only one bighorn was located on this range in January 1992 and none were detected on that range in subsequent surveys. Two groups, consisting of 12 and 19 bighorns, were captured from the Lake Mead herds and released on the Desatoya Range in 1986. These groups were released at sites four miles apart at Willow Creek and Eastgate respectively. The two groups have intermingled and are now considered to be a single population. The numbers of animals in this herd were recorded as 42 in November 1989, 71 in November 1993 and 99 in October 1995. The bighorn population on the Granite Range

was established from several populations of California bighorns including four from the Idaho Owyhee River drainage in 1980, a single adult ewe from the Nevada Hell Creek herd in January 1983, and 19 animals from Williams Lake, British Columbia in March 1983. Population estimates for the Granite Range herd were 57 in 1989, 64 in 1991, 76 in 1992, 81 in 1993, 83 in 1994, and 96 in 1995. This population has tripled since establishment, even though it was demonstrated that bighorn sheep shared biotype 3, biogroup 11 *Pasteurella* with the domestic wether which trespassed on that range.

DISCUSSION

Pasteurella spp. colonize the oropharyngeal mucosa of lambs during their first weeks of life, most likely from maternal licking and nose-to-nose contact with their dam and/or other animals. Aerosolized *Pasteurella* which can be inhaled into the lungs may also contribute to colonization (Gibbs et al., 1984, Donachie, 1995). Bighorn and domestic sheep readily intermingle when they encounter one another. We observed that when bighorn and domestic sheep were placed in adjoining pens separated by woven wire fencing, nose-to-nose contact was made and castrated males licked the noses of ewes (D. L. Hunter and A. C. S. Ward, unpubl.). Domestic sheep were observed on range in this study with or in close proximity to bighorn sheep. Considering the means of colonization and the attraction for sheep of the two species to intermingle, we conclude that transmission could have occurred by nose-to-nose contact of the two sheep species and potentially by aerosols produced if animals coughed or sneezed during periods of intermingling.

The incidence of *Pasteurella* spp. isolated from sheep in this study was higher than that reported earlier (Ward et al., 1990) and appears to have resulted, at least in part, from eliminating prolonged periods between collection and culturing of samples that occurred in the earlier study. In this study, 92% of the bighorn sheep were found to carry biotype T (*P. trehalosi*) in contrast to 34% which carried *P. haemolytica* biotype A. The prevalence of biotype A strains in domestic sheep in this study was in agreement with Ward et al.'s (1990) study of 73 domestic sheep but was higher than in a smaller group of domestic sheep (Queen et al., 1994). The incidence of biotype 3 organisms in bighorn sheep in this study was considerably higher than that detected in domestic sheep in this and previous studies (Ward et al., 1990; Queen et al., 1994).

Although phenotypically identical biotype T (*P. trehalosi*) organisms were isolated from all bighorn sheep tested, except three on the Desatoya Range, none of those from which DNA was evaluated were shared by sheep on the different ranges. Isolation of biotype T (*P. trehalosi*) strains vary for domestic sheep populations with age, season and geographic location (Frank, 1982; Gilmour and Gilmour, 1989). Similar variances may also occur in bighorn sheep, as suggested by the variances we detected between the sheep on the different ranges.

Biotype A, biogroup 1 *P. haemolytica*, particularly serotype 2 (A2), are common in both cattle and domestic sheep (Frank, 1982; Frank et al., 1994). Although the DNA profiles of the two A2 isolates recovered from domestic sheep removed from the Desatoya Range varied from that of an isolate from a bighorn, isolation of A2 organisms from the bighorn was suggestive of transmission from either domestic sheep or cattle. Biotype 3 organisms, including biogroups 3B, 3AB, and 6 were isolated from bighorn sheep on the Granite Range at the time that the domestic sheep was removed. The biotype 3, biogroup 11 *P. haemolytica* was isolated from the domestic sheep at that time, and subsequently from one bighorn 3 mo later and two additional bighorns 7 mo later. All four biogroup 11 isolates had identical REA patterns. Although DNA bands smaller than 2.0 kb generally were not distinct

enough for definitive evaluation, the identical patterns with a S_{AB} value of 1.0, above 2.0 kb, are strong evidence that the organisms were identical.

Although domestic sheep did not have indole-positive isolates which shared REA or ribotype profiles with isolates from bighorn sheep, the similarity coefficients for profiles of isolates from domestic sheep associated with the two ranges were very similar. These similarities are evidence that the isolates were very closely related and possibly representatives of a strain that is common in domestic sheep in general or domestic sheep that had previously intermingled.

These findings are evidence for heterogeneous populations of *Pasteurella* spp. on each range which differ from those carried by sheep populations on each of the other ranges. If the differences are also associated with antigenic composition, they may be associated with virulence variances. Potential pathogenic strains of *Pasteurella* spp. in one population of bighorn sheep in which the organisms are indigenous may stimulate protective antibody production in that group of sheep but pose a threat to another population of bighorn sheep when first introduced. This potential should be considered when bighorn populations are being augmented by introducing sheep from other populations such as was done to augment the population on the Tobin Range in 1991.

Although we identified only one strain of *Pasteurella* sp. that was shared by the two sheep species, we cannot conclusively state that there were not others. In an earlier study (Jaworski et al., 1993) in which transmission of one strain from adult bighorns to caesarian derived lambs was demonstrated, some of the strains carried by the adult sheep were either not transmitted to, or were not present in, high enough numbers in the lambs for detection by the same procedures used in this study. Bacterial colonies are commonly crowded on culture media and strains which are in relatively low numbers compared to predominant strains, may be missed in routine isolation procedures. Therefore, we recognize that the bighorn and domestic sheep in this study may have shared strains which were not detected. It is also possible that the biotype 3, biogroup 11 strain isolated from sheep on the Granite Range may have been present in the bighorn population before contact with domestic sheep, but was not detected.

To conclusively demonstrate that specific *Pasteurella* sp. strains were transmitted from one sheep species to the other, it would have been necessary to know the characteristics of organisms present in each group of sheep prior to contact. Since samples were not taken from any of the animals prior to intermingling, it was impossible to determine if transmission had occurred. The fact that identical isolates were cultured from both domestic and bighorn sheep on the same range is suggestive of transmission.

All bighorn sheep have disappeared from the East and Tobin Ranges since domestic sheep were detected on those ranges. None of the bighorn sheep were observed to be ill nor were any carcass sightings reported during the duration of the study. Therefore, the causes of deaths, believed associated with the loss of these populations, have not been identified.

Although disease resulting from contact of bighorn sheep with domestic sheep was not demonstrated in this study, nose-to-nose fence-line contact have resulted in transmission of a phenotypically and genotypically characterized *P. haemolytica* strain from domestic to bighorn sheep (D. L. Hunter and A. C. S. Ward, unpubl.). This organism was associated with pneumonia and death of clinically healthy bighorn sheep and domestic sheep with clinical symptoms of ovine progressive pneumonia. Although disease and transmission may not occur in all instances when bighorn sheep contact domestic sheep, recommendations for management of domestic sheep on or near bighorn range should be followed to prevent potential for trans-

mission of diseases to bighorn sheep (Bureau of Land Management, 1992).

Bighorn sheep populations are subject to many factors which may result in their population reduction. Small isolated populations with little or no interaction with other herds may lack genetic diversity which may contribute to increased susceptibility to some disease agents. We are currently conducting tests with bighorn DNA for genetic markers associated with disease resistance to identify animals with the ability to pass innate immunity to their offspring. Selective breeding favoring transmission of genes associated with resistance could contribute to increasing numbers of bighorn sheep on range.

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