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Sharing of *Pasteurella* spp. Between Free-ranging Bighorn Sheep and Feral Goats

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ABSTRACT: *Pasteurella* spp. were isolated from feral goats and free-ranging bighorn sheep (*Ovis canadensis canadensis*) in the Hells Canyon National Recreation Area bordering Idaho, Oregon, and Washington (USA). Biovariant 1 *Pasteurella haemolytica* organisms were isolated from one goat and one of two bighorn sheep found in close association. Both isolates produced leukotoxin and had identical electrophoretic patterns of DNA fragments following cutting with restriction endonuclease *Hae*III. Similarly *Pasteurella multocida multocida a* isolates cultured from the goat and one of the bighorn sheep had D type capsules, serotype 4 somatic antigens, produced dermonecrotoxin and had identical *Hae*III electrophoretic profiles. A biovariant U^{β} *P. haemolytica* strain isolated from two other feral goats, not known to have been closely associated with bighorn sheep, did not produce leukotoxin but had biochemical utilization and *Hae*III electrophoretic profiles identical to those of isolates from bighorn sheep. It was concluded that identical *Pasteurella* strains were shared by the goats and bighorn sheep. Although the direction of transmission could not be established, evidence suggests transmission of strains from goats to bighorn sheep. Goats may serve as a reservoir of *Pasteurella* strains that may be virulent in bighorn sheep; therefore, goats in bighorn sheep habitat should be managed to prevent contact with bighorn sheep. Bighorn sheep which have nose-to-nose contact with goats should be removed from the habitat.

Key words: Bighorn sheep, feral goats, *Ovis canadensis canadensis, Pasteurella* spp., pneumonia, restriction enzyme analysis.

Members of the bacterial family Pasteurellaceae are common commensals of the upper respiratory tract of wild and domestic animals (Biberstein, 1978; Mutters et al., 1989). The genus *Pasteurella* comprises a diverse group of organisms, many incriminated as primary causes of or as important contributors to diseases in numerous hosts (Biberstein, 1978). Three *Pasteurella* species, *haemolytica, trehalosi,* and *multocida,* have been most commonly associated with disease. Organisms primarily from domestic livestock, and identified as *P. haemolytica,* are now generally considered in the genus, *Mannheimia,* divided into five species, *haemolytica, granulomatis, glucosida, ruminalis,* and *varigena,* plus multiple unnamed groups (Angen et al., 1999). Another system (Jaworski et al., 1998), based on biogrouping (Bisgaard and Mutters, 1986; Bisgaard, 1995), has been used to differentiate isolates with characteristics listed for *P. haemolytica* by the Centers for Disease Control and Prevention (Weyant et al., 1995). This system, which retains the name *Pasteurella haemolytica,* has a greater capacity than that of Angen et al. (1999) to differentiate isolates into distinguishable groups based on biochemical utilization. More than 4,000 isolates, primarily from North American native ruminants, have been differentiated into more than 70 biovariant groups (Jaworski et al., 1998; Ward et al., 1999; Ward, unpubl.).

Bighorn sheep (*Ovis canadensis*) are particularly susceptible to *Pasteurella*-related pneumonia (Foreyt et al., 1994). It has been documented that *Pasteurella* spp. from domestic sheep can cause lethal disease in bighorn sheep (Onderka and Wishart, 1988; Foreyt et al., 1994). In addition a review of numerous reports of pneumonic epizootics in bighorn sheep after

contact with domestic sheep suggests transmission of disease causing organisms from domestic sheep to free-ranging bighorn sheep (Martin et al., 1996).

Domestic goats also carry a variety of *Pasteurella* strains (Midwinter et al., 1985; Viera et al., 1993; Ward et al., 2002). In this report we present evidence which suggests transmission of unique *Pasteurella* strains from feral goats to free-ranging bighorn sheep in two locations near the Hells Canyon National Recreation Area (46°10′N, 117°00′W) in Idaho, Oregon, and Washington (USA). Evaluation of isolates from animals in these locations was incidental to an epizootic of pneumonia in bighorn sheep with a high mortality rate associated with *Pasteurella.*

A feral goat was observed with a herd of bighorn sheep near the Snake River in southeastern Washington on 2 and 3 November 1995. On 29 November the goat, a bighorn ram, and a bighorn ewe were observed separated from the herd. The goat and ram appeared clinically healthy but the ewe had signs of respiratory disease, including a rapid respiration rate and nasal discharge. The three animals were shot in an attempt to prevent transmission of disease to other bighorn sheep. Necropsy of each animal was conducted immediately on site. Nasal and oropharyngeal swab samples, collected using Accu-Cul-Shure transport systems (Accumed Corporation, Pleasantville, New York, USA), and lung samples were submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL; Washington State University, Pullman, Washington) where they were cultured for bacteria using standard procedures (Carter, 1990). *Pasteurella* isolates from these animals were sent to the University of Idaho Caine Veterinary Teaching Center (CVTC; Caldwell, Idaho) for biovariant identification (Biberstein et al., 1991; Jaworski et al., 1998).

Between the time of the first observation of the feral goat and 16 April 1996, 20 free-ranging bighorn sheep were observed, either with signs of respiratory disease or dead, within a radius of approximately 30 km from where the goat was shot (Cassirer et al., 1996), henceforth identified as BHS group A. Pneumonia was determined to be the cause of death in a bighorn sheep found dead in the area on 22 November 1995. Therefore, based on previous pneumonia epizootics that caused high mortality (Cassirer et al., 1996), 72 remaining bighorn sheep north of the Grande Ronde River and west of the Snake River (BHS group B) were captured and transported to holding facilities at the Idaho Department of Fish and Game Wildlife Health Laboratory (IDFGWHL; Caldwell, Idaho) to prevent further transmission of disease. Nasal and pharyngeal swab samples from group B, and lung and liver from 64 of the animals in group B that died at the IDFGWHL were submitted to WADDL and/or CVTC.

Two additional feral goats found in Idaho, across the Snake River from the capture site of BHS group B, which appeared clinically healthy, were captured by netgun and transported to facilities at CVTC on 12 December 1995. Oropharyngeal swab samples were collected using Accu-Cul-Shure transport systems and nasal samples were collected using rayon tipped swabs supplied with Amies transport medium (Precision Dynamics Corporation, San Fernando, California, USA) at capture and submitted to CVTC.

Tissue, nasal, and oropharyngeal samples submitted to CVTC were inoculated onto Columbia blood agar (CBA) containing 5% sheep blood and with 5% bovine blood plus antibiotics selective for Pasteurellaceae (CBAA) (Jaworski et al., 1993). Culture media were incubated at 37 C with 10% added CO_2 and examined after 24 and 48 hr for growth of colonies characteristic of *Pasteurella.* A representative of each colony type was selected for further characterization by biochemical utilization tests (Biberstein et al., 1991; Jaworski et al., 1998), serotyping (Heddleston et al., 1972; Frank and Wessman, 1978), assays for production of toxins (Magyar and

TABLE 1. Characteirstics of *Pasteurella* isolates from a feral goat and two closely associated free-ranging bighorn sheep.

Animal	Sample type[a]	*Pasteurella* species	Biovariant/ subspecies[b]	Capsule/ serotype[c]	Toxin type[d]	REA profile[e]
Feral goat	OP	*P. haemolytica*	1	12	LktA	Ph1-1
	OP	*P. multocida*	*multocida a*	D:4	ToxA	PMD-1
	NA	*P. multocida*	*multocida a*	D:4	ToxA	PMD-2
Bighorn ram	LG	*P. multocida*	*multocida a*	D:3	ToxA	PMD-3
Bighorn ewe	OP	*P. multocida*	*multocida a*	D:4	ToxA	PMD-1
	OP	*P. multocida*	*multocida a*	D:4	ToxA	PMD-2
	OP	*P. multocida*	*multocida a*	D:4	none	PMD-2
	LG	*P. multocida*	*multocida a*	D:4	ToxA	PMD-1
	LG	*P. multocida*	*multocida a*	D:3	ToxA	PMD-3
	LG	*P. haemolytica*	1	1,2,7	LktA	Ph1-1

[a] OP = oropharyngeal; NA = nasal; LG = lung.
[b] Biovariants for *P. haemolytica* and subspecies for *P. multocida* were identified by biochemical utilization tests.
[c] Capsular types of *P. multocida* are identified with a capital letter; somatic antigen serotypes of *P. multocida* and *P. haemolytica* are identified with numbers.
[d] LktA = leukotoxin; ToxA = dermonecrotoxin.
[e] The restriction enzyme analysis (REA) profile designations are assigned to indicate variances when numbers differ and identical patterns when the letters and numbers for different isolates are identical.

Rimler, 1991; Silflow and Foreyt, 1994), and restriction enzyme analyses (REA) of DNA (Jaworski et al., 1993).

Two biochemically distinct *Pasteurella* types were isolated from the first goat and two bighorn sheep (Table 1). *Pasteurella haemolytica* biovariant 1 and *P. multocida multocida a* were isolated from the goat and bighorn ewe. Only *P. multocida multocida a* was isolated from the ram. The *P. haemolytica* biovariant 1 isolates from the feral goat and the bighorn ewe had identical *Hae*III (REA) profiles (Fig. 1) with similarity coefficients (SC) of 1.0 (Schmid et al., 1990), although they were identified by slide agglutination tests as serotypes 12, and a complex of 1, 2, and 7, respectively (Table 1). Eight isolates of *P. multocida multocida a* from the feral goat, the bighorn ram, and ewe, had identical biochemical utilization profiles and were sent to the National Animal Disease Center (NADC; USDA, Ames, Iowa, USA) for further characterization. All isolates were determined by gel diffusion (Rimler and Brogden, 1986) to have capsular type D, six isolates (two from the goat and four from the ewe) were serotype 4 and one each from the ram and ewe were serotype 3. Seven of the *P. multocida multocida* isolates were determined by a colony-blot assay (Magyar and Rimler, 1991) to produce dermonecrotoxin. The eight isolates produced three different *Hha*I REA profiles (Wilson et al., 1992); one goat isolate and two from the bighorn ewe had SC values of 1.0 indicating that they were identical (Fig. 2). In addition to *Pasteurella* isolates cultured from the three animals above, 22 *P. multocida multocida a* isolates cultured from bighorn sheep in groups A and B were also evaluated by NADC for capsular types and tested for dermonecrotoxin production. In addition, all were negative for the *toxA* gene and were either not capsulated or had capsular type A (Weiser et al., 2003).

Pasteurella haemolytica biovariants U^{β} and $U^{\alpha\beta}$ and *P. trehalosi* biovariant 2 were isolated from the two feral goats in Idaho sampled 12 December (Table 2). Isolates from bighorn sheep in groups A and B, including seven *P. haemolytica* biovariant U^{β} and three *P. haemolytica* biovariant $U^{\alpha\beta}$ were compared by REA with the goat isolates. Five of the bighorn sheep biovariant U^{β} isolates had REA profiles with SC values of 1.0 with isolates from the two feral goats (Fig. 3). The other two bighorn sheep U^{β} isolates produced individually distinct profiles. The three $U^{\alpha\beta}$ bighorn

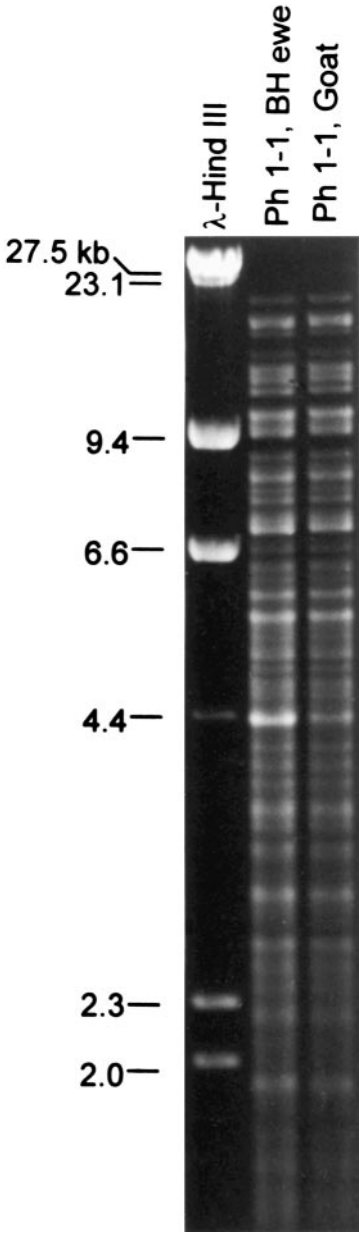

FIGURE 1. Restriction enzyme analysis (REA) of selected *P. haemolytica* biovariant 1 isolates from a bighorn ewe (BH ewe) and a feral goat (Goat). Restriction profiles (Ph1-1) were generated with *Hae*III. A similarity coefficient of 1.0 was found for the two isolates shown, indicative of identity. λ-*Hind*III marker sizes are indicated in kilobases (kb).

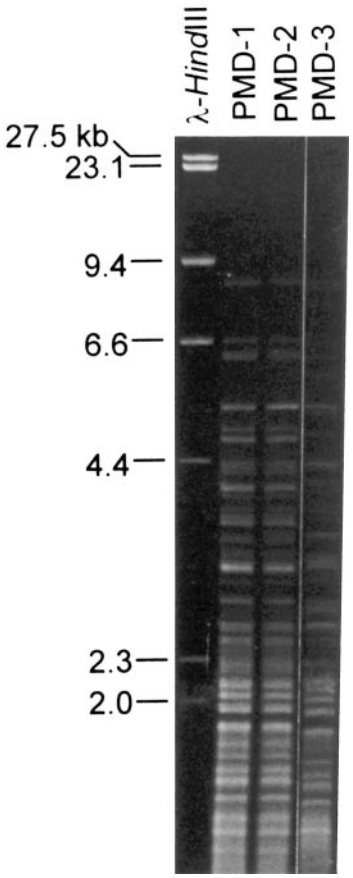

FIGURE 2. Restriction enzyme analysis (REA) of three representative *P. multocida multocida a* isolates (PMD-1, PMD-2, PMD-3) were generated with *Hha*I. With respect to isolates with the PMD-1 profile, similarity coefficients of 0.99 and 0.68 were found for PMD-2 and PMD-3 isolates, respectively. λ-*Hind*III marker sizes are indicated in kilobases (kb).

sheep isolates and the one goat $U^{\alpha\beta}$ isolate also produced distinct profiles.

Isolation of identical strains of *P. haemolytica* biovariants 1 and U^{β} and capsular type D ToxA + *P. multocida multocida a* from goats and bighorn sheep early in the epizootic caused concern that these organisms had been introduced by the goats and might have been responsible for subsequent deaths of bighorn sheep. Biovariant 1 strains are most commonly associated with domestic livestock. Such strains are considered to have a high disease potential for bighorn sheep because their neutro-

TABLE 2. Characteirstics of *Pasteurella* isolates cultured from nasal and pharyngeal swab samples from two feral goats removed from bighorn sheep habitat 12 December 1995.

Animal	Sample type[a]	*Pasteurella* species	Biovariant[b]	Serotype[c]	Toxin type[d]	REA profile[e]
Goat ID#1	NA	*P. haemolytica*	U^{β}	ND	LktA neg	U^{β}-1
	OP	*P. haemolytica*	$U^{\alpha\beta}$	ND	LktA neg	$U^{\alpha\beta}$-2
	OP	*P. trehalosi*	2	ND	NT	2-4
Goat ID#2	NA	*P. haemolytica*	U^{β}	ND	LktA neg	U^{β}-1
	NA	*P. trehalosi*	2	ND	NT	2-4
	OP	*P. haemolytica*	U^{β}	ND	LktA neg	ND

[a] NA = nasal; OP = oropharyngeal.
[b] Biovariants of *P. haemolytica* and *P. trehalosi* are identified with a number or the letter "U", with or without superscript notations.
[c] ND indicates that isolates from these animals were not serotyped.
[d] LktA neg = leukotoxin negative; NT = isolates were not tested for leukotoxin production.
[e] The restriction enzyme analysis (REA) profile designations are assigned to indicate variances when numbers differ and identical patterns when the letters and numbers for different isolates are the same; ND = not determined.

phils are reported to be highly sensitive to leukotoxin produced by tested biovariant 1 isolates (Silflow and Foreyt, 1994). In contrast biovariant U^{β} isolates have been cultured from multiple hosts including bison, moose, and domestic goats (Jaworski et al., 1998; Dyer et al., 2001; Ward et al., 2002), as well as clinically healthy bighorn sheep sampled in Arizona, Idaho, Nevada, and Canada (Ward, unpubl.) and are considered to have a relatively low disease potential for bighorn sheep. Capsular type D ToxA + *P. multocida* strains have been associated with atrophic rhinitis in swine (Chanter and Rutter, 1986) and with disease in goats (Baalsrud, 1987; Zamri-Saad et al., 1996), but their potential for causing disease in bighorn sheep is unknown.

Because samples were not obtained from the animals prior to contact, the direction of transmission could not be ascertained with certainty. The fact that identical strains of *Pasteurella,* particularly biovariant 1 *P. haemolytica,* were isolated from both goats and bighorn sheep is suggestive of transmission of the organisms from goats to bighorn sheep. However, because both the biovariant 1 and ToxA + organisms were limited to the three animals shot on 29 November 1995 and were not isolated from any of the other bighorn sheep in groups A and B, there is no evidence that those organisms were associated with subsequent disease or deaths. Although we know of no other information regarding transfer of potentially lethal *Pasteurella* spp. between domestic goats and free-ranging bighorn sheep, we believe that goats can serve as a reservoir. Thus, interactions between the two species should be avoided to prevent *Pasteurella* transmission that could negatively impact the health of bighorn sheep populations.

Pack goats have gained popularity for use on public and private lands. We recommend that individuals with pack goats have total control of their animals when in or near bighorn sheep habitat, both while on the trail and at the campsite. Likewise, we recommend that any bighorn sheep should be driven away from goats to prevent nose-to-nose contact and that any bighorn sheep that does come into direct contact should be removed from the herd to prevent potential transmission of disease causing organisms to other bighorn sheep.

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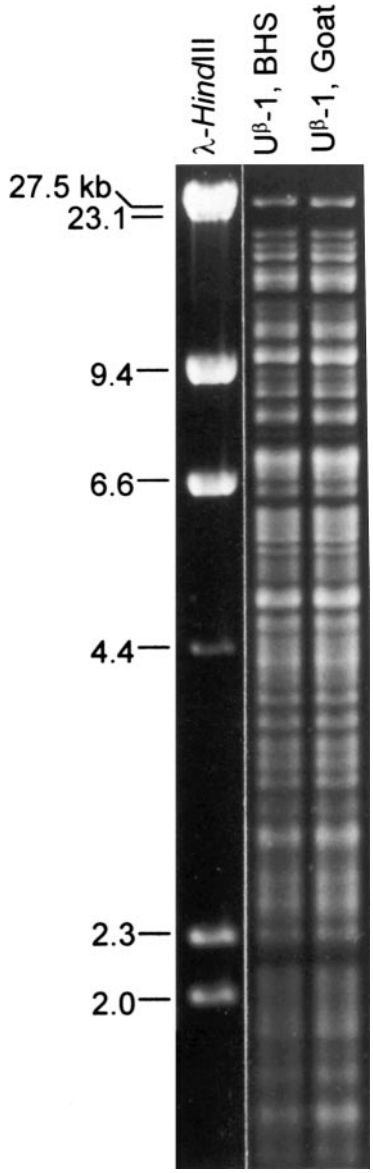

FIGURE 3. Restriction enzyme analysis (REA) of representative *P. haemolytica* biovariant U^{β} isolates from the bighorn sheep (BHS) and feral goats (Goat). Restriction profiles (U^{β}-1) were generated with *Hae*III. A similarity coefficient of 1.0 was found for the two isolates shown, indicative of identity. λ-*Hind*III marker sizes are indicated in kilobases (kb).

exceptional proficiency and skill in field observations and collection of animals and samples; G. Majors, a member of the Foundation for North American Wild Sheep, who assisted with many aspects of this investigation during the initial phase of the epizootic; and, the Oregon Hunter's Association for financial support. We thank G. Weiser for his comments and review of the manuscript. We also acknowledge the Foundation for North American Wild Sheep for their immediate support, financially and otherwise, that made this study possible.

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