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USING RIBOSOMAL RNA GENE RESTRICTION PATTERNS IN DISTINGUISHING ISOLATES OF *PASTEURELLA HAEMOLYTICA* FROM BIGHORN SHEEP (*OVIS CANADENSIS*)

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ABSTRACT: *Pasteurella haemolytica* isolates (n = 31) from two isolated captive herds of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were characterized and compared phenotypically (biotype, serotype, hemolytic activity) and by a genomic fingerprinting method known as ribotyping. Seven to nine distinct phenotypes were observed. Depending on the method used for serotyping, one to three phenotypes were common to both herds. Eighteen isolates, recovered from both herds, were non-hemolytic, biotype T, indirect hemagglutination assay serotype 4. Ribotyping, a method for highlighting genetically conserved deoxyribonucleic acid restriction site heterogeneity with a ^{32}P-labelled *Escherichia coli* ribosomal ribonucleic acid probe, produced six to eight distinct ribotype pattern groups within the 31 *P. haemolytica* isolates, depending on the restriction enzyme used. In contrast to phenotypes, ribotypes appeared unique to each herd, and ribotyping helped to further differentiate some isolates of the same biotype and serotype. In addition, ribotyping provided an alternative means for evaluating relationships between isolates differing in hemolytic activity but which were otherwise phenotypically identical. We propose that ribotyping may be a useful adjunct to other bacterial characterization methods in studying the epizootiology of pasteurellosis in bighorn sheep.

Key words: *Pasteurella haemolytica*, bighorn sheep, *Ovis canadensis*, fingerprinting, ribotyping, DNA, serotyping.

INTRODUCTION

Pasteurella haemolytica is an important primary and opportunistic pathogen of domestic and wild sheep. Respiratory disease attributed to *P. haemolytica* has adversely affected population performance in some herds of bighorn sheep (*Ovis canadensis*) in the western United States and Canada (Buechner, 1960; Foreyt and Jessup, 1982; Onderka and Wishart, 1984; Spraker et al., 1984). Although *P. haemolytica* can cause severe bronchopneumonia, it also can be isolated from clinically normal bighorn sheep (Post, 1962; Thorne, 1982; Onderka and Wishart, 1984, 1988; Dunbar et al., 1990b; Wild and Miller, 1991). A prerequisite to understanding the epizootiology of *P. haemolytica* in bighorn sheep is the ability to discern phenotypic and/or genotypic differences that may occur in isolates from various sources.

Currently, epizootiological investigations of *P. haemolytica* in bighorn sheep and other animals rely on use of biotyping and serotyping to characterize isolates (Biberstein et al., 1960; Biberstein and Gills, 1962; Biberstein, 1978). This approach has been valuable in differentiating isolates, but has potential limitations. Biotyping and serotyping rely on phenotypic traits, characteristics possibly under the control of unknown environmental influences (Brown and Williams, 1985). Moreover, the sensitivity of these tests may be inadequate to differentiate strains reliably, because more than one strain may make up a given serotype (Snipes et al., 1989, 1990). Genomic, or chromosomal deoxyribonucleic

TABLE 1. Characteristics of *P. haemolytica* isolated from captive bighorn sheep from Washington (n = 17) and Colorado (n = 14) (USA).

Isolate number	Animal number	Site of origin	Biotype	RPA[a] serotype	IHA[b] serotype	Hemolysis	*Eco*RI ribotype	*Hin*cII ribotype
				Washington				
30	R24	Lung	T	4	4	+	3	1
31	B27	Lung	T	4	4	+	2	1
32	G39	Lung	T	3, 4, 10	3, 10	+	15	2
33	G39	Lung	A	2	2	+	5	3
34	OR41	Lung	A	2	2	+	5	3
35	OR41	Lung	T	10	3, 10	+	15	2
36	OR41	Nares	A	2	2	+	5	3
37	OR41	Lung	T	3, 4, 10	3, 10	+	15	2
87	2184	Lung	T	3, 4, 10	4	−	3	1
88	2214	Lung	T	4	4	−	2	1
89	OR2	Nares	T	4	4	−	3	1
91	OR44	Nares	T	3, 4, 10	3, 10	−	32	4
92	R11	Nares	T	3, 4	4	−	4	1
93	Y10	Nares	T	3, 4, 10	4	−	3	1
94	R12	Nares	T	3, 4, 10	4	−	4	1
95	Y7	Lung	T	4	4	−	2	1
97	R5	Lung	T	4	4	−	2	1
				Colorado				
50	Q889	Nares	T	4	4	−	1	5
51	Q889	Tonsil	T	3, 4	4	−	1	5
52	Q889	Bronchus	T	3, 4	4	−	1	5
53	Q889	Nares	T	3, 4	4	−	1	5
54	Q889	Tonsil	T	3, 4	4	−	1	5
76	T89	Bronchus	T	4	4	−	1	5
77	T82	Tonsil	T	3, 4, 10	NT[c]	−	11	6
78	A588	Tonsil	T	4	4	−	1	5
80	E88	Tonsil	T	4	4	−	1	5
81	G78	Tonsil	T	NT	4	−	1	5
82	H83	Tonsil	T	3	3	−	11	6
83	L88	Tonsil	T	NT	3	−	11	6
84	M86	Tonsil	T	3, 4, 10	3	−	11	6
86	T88	Tonsil	T	4	4	−	1	5

[a] RPA = Rapid plate agglutination.
[b] IHA = Indirect hemagglutination.
[c] NT = Not typable.

acid (DNA), fingerprinting of bacteria offers a valuable adjunct to phenotypic characterization techniques, and the use of nucleic acid probes to highlight DNA restriction site heterogeneity has facilitated this procedure (Grimont and Grimont, 1986; Stull et al., 1988; Tompkins et al., 1986).

The objective of this study was to determine if a genomic fingerprinting technique known as ribotyping was capable of differentiating *P. haemolytica* strains isolated from bighorn sheep. Additionally, we wanted to compare results of ribotyping with traditional serotyping results, and explore whether ribotyping could offer a new tool for clarifying the epizootiology of pasteurellosis in bighorn sheep.

MATERIALS AND METHODS

Thirty-one randomly selected *P. haemolytica* isolates from 23 individual captive bighorn sheep in two herds were examined. Fourteen isolates (numbers 50 to 86; Table 1) were obtained from captive bighorn sheep at the Colorado Division of Wildlife's Foothills Wildlife Research Facility (Fort Collins, Colorado USA). Seventeen ad-

ditional isolates (numbers 30 to 37 and 87 to 97; Table 1) were from captive bighorn sheep housed at Washington State University (Pullman, Washington USA). Isolates were obtained from nares, tonsils, or bronchi (Wild and Miller, 1991) and nares or lung (Foreyt, 1989) of apparently healthy and pneumonic bighorn sheep. Complete histories of both herds with respect to pasteurellosis are described elsewhere (Foreyt, 1989; Silflow, 1988; Wild and Miller, 1991; Miller et al., 1991).

Isolates were confirmed to be *P. haemolytica* by standard biochemical tests (Carter, 1984). Specifically, isolates were determined to produce typical reactions following inoculation of triple sugar iron agar slants (acid slant and butt, no gas), to be indole negative (spot test on filter paper impregnated with 1% 4-dimethylaminocinnamaldehyde), to be oxidase positive (spot test on filter paper impregnated with 1% tetramethyl-p-phenylenediamine), to be urease negative, and to grow on MacConkey's agar. Hemolysis on 5% bovine or other blood agar was not a required isolate characteristic for inclusion in the study (Onderka and Wishart, 1984; Onderka et al., 1988). Hemolytic activity of each isolate was determined following incubation on 5% bovine and ovine blood agar plates at 37 C for 24 hr.

Biotypes (A vs. T) were determined by inoculation of Bromcresol purple broths containing arabinose, trehalose, xylose, salicin, mannitol, and lactose, and observing for fermentation as described by Biberstein (1978). Serotypes of isolates were determined by rapid plate agglutination (RPA) (Frank and Wessman, 1978), and by indirect hemagglutination (IHA) (Biberstein et al., 1960). All RPA serotyping was performed by the National Animal Disease Center (Ames, Iowa, USA) or by the Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA). A particular phenotype of *P. haemolytica* was defined as a unique combination of biotype, serotype, and ability to lyse bovine red blood cells.

The DNA from each isolate was obtained and purified using the method of Wilson (1987). Bacterial cells were lysed with sodium dodecyl sulfate, proteins and other cellular debris were removed by digestion with proteinase-K and precipitation with hexadecyltrimethyl ammonium bromide, and DNA was precipitated with isopropanol.

In order to determine the optimal restriction enzymes to use for digesting *P. haemolytica* DNA for subsequent determination of ribotype, purified DNA (2.0 to 3.0 μg) from one biotype A isolate and one biotype T isolate was digested according to the manufacturer's directions with 10 units each of 14 different restriction enzymes in a 30 μl volume containing the appropriate buffer. Restriction enzymes evaluated were: *Eco*RI, *Pst*I, *Xho*I, *Hae*III, *Eco*RV, *Hin*dIII, *Bgl*II, *Hpa*I, *Bst*EII, *Kpn*I, *Sst*I, *Hin*cII, and *Cla*I (GIBCO BRL, Grand Island, New York, USA); and *Ban*II (New England Biolabs, Beverly, Massachusetts, USA). Optimal endonucleases were chosen based on the number and distribution of restriction fragments hybridizing with the ribosomal ribonucleic acid (rRNA) probe.

Purified DNA (2.0 to 3.0 μg) from each of the 31 *P. haemolytica* isolates was digested for 2 hr at 37 C with approximately 10 units of the restriction enzymes *Eco*RI or *Hin*cII in a 30 μl reaction mixture containing the appropriate buffer. Following digestion, samples were subject to electrophoresis in a 0.7% horizontal slab agarose gel and stained with ethidium bromide (Snipes et al., 1989).

Digested DNA restriction fragments separated by electrophoresis were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, New Hampshire, USA) using methods of Southern (1975). After transfer of DNA, blots were dried at 80 C in a vacuum oven and stored at room temperature.

Escherichia coli rRNA (Sigma Chemical Co., St. Louis, Missouri, USA) was labelled with ^{32}P and hybridized with restriction enzyme-digested and blotted *P. haemolytica* DNA using techniques previously described for *P. multocida* (Snipes et al., 1989). Specific activity of the probe was approximately 10^7 counts per minute (cpm)/μg of RNA, and blots were hybridized with approximately 10^5 to 10^6 cpm/ml of hybridization solution. Extent of hybridization of the probe with blotted DNA was analyzed by autoradiography (Snipes et al., 1989), and profiles of the 31 *P. haemolytica* isolates were compared visually and grouped based on restriction fragment patterns.

Repeatability and stability of hybridization patterns defining a ribotype were determined by subculturing a bighorn sheep biotype T isolate of *P. haemolytica* for 19 passages on bovine blood agar plates over a period of 10 wk. Ribotype was determined for each of the 19 passages following digestion of purified DNA with *Eco*RI. Prior to beginning passage of the strain, ribotypes of ten separate colonies from the stock culture also were determined following digestion of DNA with *Eco*RI.

RESULTS

Phenotypic characteristics varied both within and between sources for the 31 *P. haemolytica* isolates examined (Table 1). When serotypes were determined by RPA,

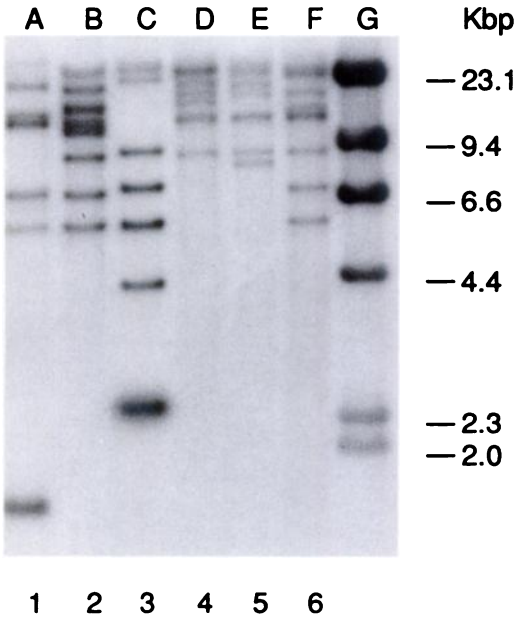

FIGURE 1. Autoradiograph of Southern blot of *Hin*cII-digested DNA from six *Pasteurella haemolytica* isolates after hybridization with a ^{32}P-labelled *E. coli* rRNA probe. Patterns in lanes A–F represent the six different ribotype groups observed using *Hin*cII. *Hin*cII ribotype designations are noted across the bottom of the figure. Lane G contains DNA isolated from lambda phage, digested with *Hin*dIII, and hybridized with *Hae*III-digested lambda phage probe. Numbers on the right represent the number of kilobase pairs (kbp) of the respective lambda phage fragments.

nine different phenotypes were identified; nonhemolytic biotype T and serotypes 4; 3, 4; and 3, 4, 10 predominated. When serotypes were determined by IHA, seven distinct phenotypes were identified; nonhemolytic biotype T, serotype 4 predominated. Only three isolates were biotype A, and all were serotype 2 by RPA and IHA. Most isolates (23/31) were non-hemolytic, and all hemolytic isolates were from one herd (Washington). Three phenotypes (non-hemolytic T4; T3, 4; and T3, 4, 10) were common to both herds when RPA was used; one phenotype (non-hemolytic T4) was common when IHA was used. Isolates that reacted with multiple typing sera in the RPA test usually reacted with fewer sera in the IHA test. For example, within isolates from the Washington herd, RPA serotypes 3, 4, 10 reduced to either 3, 10 or 4 when tested by IHA; Colorado RPA serotype 3, 4 isolates only reacted with IHA serotype 4 antisera.

The *E. coli* rRNA probe hybridized with a variable number of blotted fragments of biotype A and biotype T *P. haemolytica* DNA produced by digestion with the 14 restriction enzymes. Usually, ≤8 fragments contributed to hybridization patterns or ribotypes. Restriction enzymes *Eco*RI and *Hin*cII were chosen for use in further study, based on number (average 6 to 7) and distribution (dispersed between approximately 23.1 and 2.0 kilobase pairs) of hybridized fragments contributing to a ribotype.

Following digestion with *Hin*cII, six distinct ribotype patterns (groups) were observed among the 31 isolates (Fig. 1; Table 1). Digestions with *Eco*RI produced eight distinct ribotype groups (Fig. 2; Table 1). Study isolates conformed to the same ribotype grouping regardless of restriction enzyme used, except that *Eco*RI further subdivided isolates from one *Hin*cII ribotype into three separate but similar ribotypes. Isolates from Colorado bighorn sheep were designated as *Eco*RI ribotypes 1 and 11; those from Washington were designated as *Eco*RI ribotypes 2, 3, 4, 5, 15, and 32.

Using *Eco*RI for DNA digestion, ten colonies examined from one *P. haemolytica* biotype T isolate were identified as ribotype 1. Subsequent isolates from 19 laboratory passages over a 10-week-period continued to be identified as ribotype 1.

Relationships between RPA and IHA serotypes and ribotype are summarized in Table 2. Using RPA, multiple serotypes were revealed within a single ribotype as well as multiple ribotypes within a single serotype. In contrast, using IHA, multiple ribotypes comprised a single serotype, but not vice-versa.

Combining biotype, IHA serotype, and hemolytic activity with *Eco*RI ribotypes, 11 distinct phenotype/genotype combinations were observed (Table 3). Among

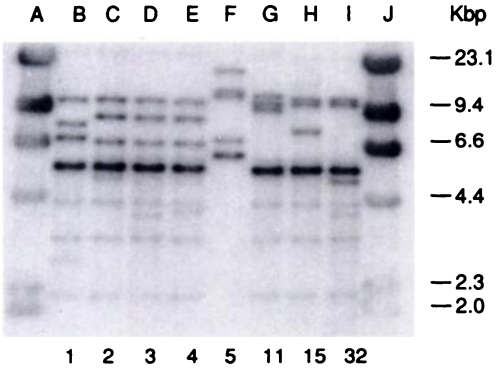

FIGURE 2. Autoradiograph of Southern blot of *Eco*RI-digested DNA from eight *Pasteurella haemolytica* isolates after hybridization with an *E. coli* rRNA probe. Patterns in lanes B–I represent the eight different ribotypes observed using *Eco*RI. *Eco*RI ribotype designations are noted across the bottom of the figure. Lanes A and J contain the lambda phage standard as described for Figure 1. Numbers on the right represent the number of kilobase pairs (kbp) of the respective lambda phage fragments.

these, no overlap existed between isolates from the two separate herds of bighorn sheep.

DISCUSSION

When isolates of *P. haemolytica* examined in this study were serotyped, combination reactions were observed, particularly when serotype was determined by RPA. Such combinations are not unusual with *P. haemolytica* isolated from bighorn sheep (Dunbar et al., 1990a).

As applied here, ribotyping proved to be a relatively simple and repeatable laboratory technique that provided a useful adjunct for characterizing *P. haemolytica* from bighorn sheep. Because ribotyping evaluates genotype rather than phenotype, it contributes unique information not available using traditional biotyping and serotyping techniques. Biotyping and serotyping rely on phenotypic traits. These may be inconsistently expressed, influenced by environmental factors such as temperature or growth media composition (Brown and Williams, 1985).

Ribotyping, a method for highlighting DNA restriction site heterogeneity with a rRNA probe, compares highly conserved rRNA genes and associated sequences not subject to frequent mutation (Grimont and Grimont, 1986). Ribotyping has been previously shown to provide a useful tool for more in-depth epizootiologic studies of *P. multocida* infection in fowl as well as other animals (Snipes et al., 1989, 1990). The stability and repeatability of ribotyping *P.*

TABLE 2. *Eco*RI ribotypes comprising different biotypes and serotypes of *Pasteurella haemolytica* from bighorn sheep from Washington and Colorado.

Biotype + RPA[a] serotype	*Eco*RI ribotypes	Number of isolates
A2	5	3
T3	11	1
T4	1, 2, 3	11
T10	15	1
T3, 4	1, 4	5
T3, 4, 10	3, 4, 11, 15, 32	8
T/NT[b]	1, 11	2
Biotype + IHA[c] serotype	**EcoRI ribotypes**	**Number of isolates**
A2	5	3
T3	11	3
T4	1, 2, 3, 4	20
T3, 10	15, 32	4
T/NT	11	1

[a] RPA = Rapid plate agglutination.
[b] NT = Not typable.
[c] IHA = Indirect hemagglutination.

TABLE 3. Summary of phenotype and genotype profiles of *Pasteurella haemolytica* from bighorn sheep from Washington and Colorado.

Biotype	IHA[a] serotype	Hemolysis	*Eco*RI ribotype	Number of isolates
Washington: isolates				
A	2	+	5	3
T	4	+	2	1
T	4	+	3	1
T	4	−	2	3
T	4	−	3	3
T	4	−	4	2
T	3, 10	+	15	3
T	3, 10	−	32	1
Colorado: isolates				
T	4	−	1	10
T	3	−	11	3
T	NT[b]	−	11	1

[a] IHA = Indirect hemagglutination.
[b] NT = Not typable.

haemolytica were confirmed by consistent identification of *Eco*RI ribotype from stock strain replicates and isolates after multiple passages. In previous studies with digested and blotted *P. multocida* DNA, identical ribotype patterns were produced using either radiolabelled *P. multocida* rRNA or *E. coli* rRNA as a probe (K. Snipes, unpublished data). Thus, *E. coli* rRNA provides an inexpensive, commercially available probe.

Comparing ribotyping results with results from standard biotyping and serotyping techniques and hemolysis determinations provided several noteworthy observations. These comparisons demonstrated the potential utility of this technique in helping to fingerprint *P. haemolytica*, and therefore study the epizootiology of pasteurellosis in bighorn sheep.

Ribotyping enabled the characterization and grouping of isolates in this study which were untypable by serotyping, presumably either because of lack of capsule or appearance of a novel serotype (Aarsleff et al., 1970). In addition, ribotyping provided a means for comparing isolates of the same biotype and serotype that differed only in ability to lyse bovine and ovine red blood cells or to produce detectable hemolysis. Ribotyping was particularly useful in examining relatedness of some isolates from the Washington herd that differed in this respect.

In comparing ribotype and serotype data, relationships were more apparent when IHA was used. This was particularly true for the biotype T isolates. Using RPA, multiple ribotypes were observed to comprise a single serotype, but multiple serotypes also were found to be of the same ribotype. Using IHA, only multiple ribotypes within a given serotype were observed. In the latter case, ribotyping provided a tool for further characterizing and differentiating isolates of the same serotype. From the results we inferred the existence of multiple strains within a given serotype.

When IHA phenotypes and ribotypes were combined and differences in hemolytic activity considered, eleven distinct combinations were obtained, with some appearing more closely related than others. Methods for determining the precise degree of relatedness (percent similarity) between these profiles are being developed. At present, we are adopting a conservative approach regarding classifying distinct biotype, serotype, and ribotype profiles as different isolates. Using these criteria, no complete isolate profiles were observed to be shared between isolates from the two herds in this study. It follows that ribotyping may aid in clarifying the epizootiology of pasteurellosis at the herd level.

Additional bighorn isolates from a variety of geographic locations need to be examined to further evaluate ribotyping in relation to biotyping and serotyping. Based on our findings, we believe that ribotyping is a useful technique, either alone or in conjunction with IHA serotyping, for characterizing *P. haemolytica* in epizootiologic studies. Comparison of ribotypes present in isolated and mixed herds of bighorn sheep, as well as domestic livestock, may aid in tracking transmission of *P. haemolytica* within and among species. Such information is critical to formulation of effective, equitable management practices.

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LITERATURE CITED

AARSLEFF, B., E. L. BIBERSTEIN, B. J. SHREEVE, AND D. A. THOMPSON. 1970. A study of untypable strains of *Pasteurella haemolytica*. Journal of Comparative Pathology 80: 493–498.

BIBERSTEIN, E. L. 1978. Biotyping and serotyping *Pasteurella haemolytica*. *In* Methods in microbiology, Vol. 10, T. Bergen and J. R. Norris (eds.). Academic Press, New York, New York, pp. 253–269.

———, AND M. G. GILLS. 1962. The relation of antigenic types to the A and T types of *Pasteurella haemolytica*. Journal of Comparative Pathology and Therapeutics 72: 316–320.

———, ———, AND H. D. KNIGHT. 1960. Serologic types of *Pasteurella haemolytica*. Cornell Veterinarian 50: 283–300.

BROWN, M. R. W., AND P. WILLIAMS. 1985. The influence of environment on envelope properties affecting survival of bacteria in infections. Annual Review of Microbiology 39: 527–556.

BUECHNER, H. K. 1960. The bighorn sheep in the United States, its past, present, and future. Wildlife Monographs 4: 1–74.

CARTER, G. R. 1984. Genus 1 *Pasteurella*. *In* Bergey's manual of systematic bacteriology, Vol. 1, N. R. Krieg (ed.). Williams and Wilkins, Baltimore, Maryland, pp. 552–558.

DUNBAR, M. R., A. C. S. WARD, K. G. EYRE, AND M. BULGIN. 1990a. Serotypes of *Pasteurella haemolytica* in free ranging Rocky Mountain bighorn sheep. Biennial Symposium of the Northern Wild Sheep and Goat Council 7: 102–108.

———, ———, AND G. POWER. 1990b. Isolation of *Pasteurella haemolytica* from tonsillar biopsies of Rocky Mountain bighorn sheep. Journal of Wildlife Diseases 26: 210–213.

FOREYT, W. J. 1989. Fatal *Pasteurella haemolytica* pneumonia in bighorn sheep after direct contact with clinically normal domestic sheep. American Journal of Veterinary Research 50: 341–344.

———, AND D. A. JESSUP. 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. Journal of Wildlife Diseases 18: 163–168.

FRANK, G. H., AND G. R. WESSMAN. 1978. Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. Journal of Clinical Microbiology 7: 142–145.

GRIMONT, F., AND P. A. D. GRIMONT. 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Annales Institute Pasteur/Microbiologie 137B: 155–163.

MILLER, M. W., N. T. HOBBS, AND E. S. WILLIAMS. 1991. Spontaneous pasteurellosis in captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): Clinical, laboratory, and epizootiological observations. Journal of Wildlife Diseases 27: 534–542.

ONDERKA, D. K., AND W. D. WISHART. 1984. A major bighorn sheep die-off from pneumonia in southern Alberta. Biennial Symposium of the Northern Wild Sheep and Goat Council 4: 356–363.

———, AND ———. 1988. Experimental contact transmission of *Pasteurella haemolytica* from clinically normal domestic sheep causing pneumonia in Rocky Mountain bighorn sheep. Journal of Wildlife Diseases 24: 663–667.

———, S. A. RAWLUK, AND W. D. WISHART. 1988. Susceptibility of Rocky Mountain bighorn sheep and domestic sheep to pneumonia induced by bighorn and domestic livestock strains of *Pasteurella haemolytica*. Canadian Journal of Veterinary Research 52: 439–444.

POST, G. 1962. Pasteurellosis of Rocky Mountain bighorn sheep (*Ovis canadensis*). Journal of Wildlife Diseases 23: 1–14.

SILFLOW, R. M. 1988. Comparison of pulmonary host defense mechanisms in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) and domestic sheep. M.S. Thesis. Washington State University, Pullman, Washington, 108 pp.

SNIPES, K. P., D. C. HIRSH, R. W. KASTEN, L. M. HANSEN, D. W. HIRD, T. E. CARPENTER, AND R. H. MCCAPES. 1989. Use of an rRNA probe and restriction endonuclease analysis to fingerprint *Pasteurella multocida* isolated from turkeys and wildlife. Journal of Clinical Microbiology 27: 1847–1853.

———, ———, ———, T. E. CARPENTER, D. W. HIRD, AND R. H. MCCAPES. 1990. Differentiation of field isolates of *Pasteurella multocida* serotype 3, 4 from live vaccine strain by genotypic characterization. Avian Diseases 34: 419–424.

SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98: 503–517.

SPRAKER, T. R., C. P. HIBLER, G. G. SCHOONVELD, AND W. S. ADNEY. 1984. Pathologic changes and microorganisms found in bighorn sheep during a stress-related die-off. Journal of Wildlife Diseases 20: 319–327.

STULL, T. L., J. J. LIPUMA, AND T. D. EDLIND. 1988. A broad-spectrum probe for molecular epidemiology of bacteria: Ribosomal RNA. Journal of Infectious Diseases 157: 280–286.

THORNE, E. T. 1982. Pasteurellosis. *In* Diseases of wildlife in Wyoming, 2nd ed., E. T. Thorne, N. Kingston, W. R. Jolley, and R. C. Bergstrom (eds.). Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 72–77.

TOMPKINS, L. S., N. J. TROUP, A. LABIGNE-ROUSSEL, AND M. L. COHEN. 1986. Cloned, random chromosomal sequences as probes to identify *Salmonella* species. Journal of Infectious Diseases 154: 156–162.

WILD, M. A., AND M. W. MILLER. 1991. Detecting nonhemolytic *Pasteurella haemolytica* infections in healthy Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): Influences of sample site and handling. Journal of Wildlife Diseases 27: 53–60.

WILSON, K. 1987. Preparation of genomic DNA from bacteria. *In* Current protocols in molecular biology, Vol. 1, F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (eds.). John Wiley and Sons, New York, New York. pp. 2.4.1–2.4.2.

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