JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 831–835
0095-1137/93/040831-05$02.00/0

Vol. 31, No. 4

Use of DNA Analysis of *Pasteurella haemolytica* Biotype T Isolates To Monitor Transmission in Bighorn Sheep (*Ovis canadensis canadensis*)†

M. D. JAWORSKI,[1] A. C. S. WARD,[1]* D. L. HUNTER,[2] AND I. V. WESLEY[3]

Department of Animal and Veterinary Science, Caine Veterinary Teaching and Research Center, College of Agriculture, University of Idaho, 1020 East Homedale Road,[1] and Idaho Department of Fish and Game,[2] Caldwell, Idaho 83605, and National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010[3]

Received 18 September 1992/Accepted 7 January 1993

Pneumonia has been identified as a major cause of poor lamb survival in indigenous herds of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in central Idaho. *Pasteurella haemolytica* was isolated from five adult Rocky Mountain bighorn ewes captured from a free-ranging herd in central Idaho. The lambs from two of these ewes delivered by cesarean section were free of *P. haemolytica* until 40 days of age and after repeated contact with their dams. The lambs subsequently developed signs of pneumonia, and *P. haemolytica* was isolated from nasal, pharyngeal, and transtracheal wash samples from each lamb. All *P. haemolytica* biotype T isolates from the ewes and lambs, as well as those from a 9-month-old lamb of the same herd from which samples for culture were obtained 2 years earlier, were subjected to *Hae*III restriction enzyme analysis (REA) and ribotyping. Two ribotypes and seven REA patterns were visually distinguishable by these procedures. Similarity coefficients (S_{AB}) of 0.09 to 0.95 were calculated for the seven REA patterns. The REA patterns of the isolates from the lambs were identical (S_{AB} = 1.0). The isolates from the lambs also had S_{AB} values of 1.0, which was indicative of identity with one of the seven isolates cultured from the ewes at the time of capture and with the organism isolated from the 9-month-old lamb. These procedures have the discriminatory capabilities necessary to monitor the transmission of specific strains of bacteria within and between animal populations.

Lamb mortality has exceeded 90% each year since 1988 in some central Idaho herds of bighorn sheep (*Ovis canadensis canadensis*). Observations of free-ranging bighorn sheep in 1990 provided evidence that pneumonia associated with *Pasteurella* infections may have contributed to the high lamb mortality (1). *Pasteurella* spp., which are common commensal organisms on the mucous membranes of the upper respiratory tract of numerous animal species (4, 5, 7, 15), have been isolated from the tonsils and upper respiratory tracts of clinically normal bighorn sheep (37, 41). However, *P. haemolytica* has also been incriminated as a cause of disease in various animals including bighorn sheep (5, 7, 14, 16, 18, 21, 23, 28, 29). These organisms generally act as opportunistic pathogens that can cause pneumonia when *Mycoplasma* spp., viruses, parasites, or various stress factors reduce the normal protective mechanisms of the respiratory tract (16, 24, 25).

The *Pasteurella* genus includes a diverse group of bacteria which has been divided into species, biotypes, biogroups, and serotypes. Biotypes and biogroups are differentiated on the basis of reactions of biochemical profiles in media containing different substrates (3, 8, 10, 26). *Pasteurella haemolytica* has been divided into three biotypes, biotypes A, T, and 3 (26). The serotypes of the isolates within the biotypes can be determined by detection of specific antigens produced by the bacteria (19). Certain biotypes and serotypes appear to have a predilection for particular animal species and vary in their pathogenicities (6, 17, 29, 36). For example, *P. haemolytica* biotype A serotypes 1 and 2 (A1 and A2, respectively) both colonize the nasopharyngeal mucosae of cattle, but it is serotype A1 that is predominantly associated with pneumonia in cattle (18). In contrast, pneumonia in sheep is generally associated with serotype A2, which is a common commensal organism on the nasal mucosae and with biotype T strains which are harbored in the tonsillar crypts of clinically normal sheep (2, 21). Specific host predilection and disease associations have not been reported for *P. haemolytica* biotype 3 organisms. Factors associated with host predilection of biochemically and serologically distinct bacterial strains have not been clearly identified. Markers which can be used to identify strains of *Pasteurella* associated with disease and to monitor their transmission would help to elucidate the factors associated with predilection.

Reproducible genetic markers can be identified by application of rRNA restriction fragment length polymorphisms (ribotyping) and restriction enzyme analysis (REA) (13, 20, 22). These procedures have been used in human and veterinary epidemiology to identify unique bacterial strains and trace infections to their sources (9, 12, 27). Application of these procedures to isolates of *Pasteurella* from bighorn sheep would make it possible to monitor the distributions of unique strains, gain information regarding the associations of different strains with disease, and identify routes of transmission.

The purpose of this report is to present evidence of transmission of a strain of *P. haemolytica* associated with pneumonia in bighorn sheep.

* Corresponding author.

† Published with the approval of the Director of the Idaho Agricultural Experiment Station, Moscow, Idaho, as Research Paper no. 92A09.

MATERIALS AND METHODS

Animals. Five bighorn ewes (*O. canadensis canadensis*)—identified as ewes 8, 9, 10, 11, and 12—were captured from a free-ranging herd in central Idaho on 11 April 1991 (38). Two pregnant ewes, ewes 8 and 12, which were subsequently used in evaluations to determine the cause of lamb mortality, were transported approximately 358 km to facilities near the University of Idaho Caine Veterinary Teaching and Research Center. Since the ewes did not adapt well to captivity and remained extremely excitable, the decision was made to deliver the lambs identified as 17 and 18 by cesarean section. The lambs were subsequently isolated from other animals until the lambs were 20 days of age. They were then placed in pens with their mothers for 1 to 4 h/day on postdelivery (PD) days 20, 22, 28, 29, 34, 35, 37, and 44 to allow them to acquire the microbial flora from their dams.

The lambs were fed canned evaporated milk, pasteurized domestic sheep milk, and a lamb milk replacer (Land O'Lakes, Inc., Fort Dodge, Iowa) to minimize the potential for transmission of *Pasteurella* spp. from other animals. Free access to water and mixed grass and alfalfa hay was provided beginning at 2 weeks PD. Rectal temperatures were monitored twice daily, at feeding times, to detect febrile responses associated with disease.

Samples. Nasal and tonsillar samples were collected from all five of the ewes at the time of capture and from the mothers of the cesarean-delivered lambs at the time of surgery and at selected intervals as described previously (38). Nasal and tonsillar swab samples were collected from the lambs on PD days 12, 34, 40, 53, 55, 64, 72, and 73. Transtracheal wash samples were collected from lambs on PD days 55 and 73, when signs of pneumonia were evident. In addition, lung tissue was collected from a lamb in the ewes' herd of origin (lamb 1); the lamb died at the time of capture 2 years earlier.

All samples for bacteriological cultures were inoculated onto the following three media: Columbia blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) with 5% ovine blood, Columbia blood agar with 5% bovine blood and antibiotics (5 μg of vancomycin, 5 μg of neomycin, 100 u of nystatin, and 100 μg of cycloheximide [Sigma Chemical Co., St. Louis, Mo.] plus 50 μg of sodium azide and 1 μg of thiamine monophosphate [Sigma] per ml) to provide selectivity for *Pasteurella* spp. (39), and Hayflick's agar selective for *Mycoplasma* spp. (35). The cultures were incubated at 35°C in an atmosphere with 5% added CO_2. Identification and biotyping of *Pasteurella* isolates were conducted by previously established procedures (4, 10, 26). Serotyping of the *P. haemolytica* isolates was conducted by slide agglutination tests with specific antisera obtained from G. H. Frank, National Animal Disease Center (19).

DNA isolation from bacterial cultures. DNA was isolated by a revised method of Gebhart et al. (20). A single colony was inoculated into Trypticase soy broth without glucose and was grown overnight (16 to 20 h) at 35°C. Bacteria were washed once with phosphate-buffered saline and were suspended in STE-sucrose (10 mM NaCl, 50 mM Tris [pH 8.0], 50 mM EDTA, 25% sucrose). An aliquot of this suspension was transferred to a screw-cap tube and brought to a 5-ml volume with STE-sucrose to obtain a final suspension which approximated an optical density of 0.4 at 610 nm. Proteinase K and sodium lauryl sarcosyl were added to final concentrations of 0.1 mg/ml and 1%, respectively. The resulting suspension was mixed several times by inversion and was incubated at 55°C for 3 h. Ammonium acetate was added to a final concentration of 2 M, and the suspension was incubated on ice for 20 min. Phenylmethylsulfonyl fluoride was added to a final concentration of 29 μg/ml, and the suspension was incubated on ice for an additional 20 min. The DNA was precipitated by the addition of 2 volumes of ethanol, collected by spooling onto a glass rod, and washed with 70% ethanol. The DNA was resuspended in TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA), and the nucleic acid concentration was estimated spectrophotometrically (260 nm). In addition to this method, the DNA from selected samples was also isolated by the use of CsCl density gradients (40).

REA and gel electrophoresis. Several restriction enzymes (*Hae*III, *Hha*I, *Hin*dIII, *Pst*I, *Bgl*II, and *Eco*RI) were screened. Digestion of DNA from *Pasteurella* isolates with *Hae*III gave the best resolution and discrimination among isolates. Therefore, *Hae*III digestion was used for all isolates evaluated in the present study.

The isolated DNA (3 to 6 μg) was cut with *Hae*III in a 200-μl volume for 4 h in buffers supplied by the manufacturer (International Biotechnology Incorporated, New Haven, Conn.). DNA was precipitated from the reaction mixture by the addition of 80 μl of ammonium acetate (7.5 M) and 560 μl of ethanol. Restriction fragments were size separated in 0.6% agarose (30 V, 20 h) with TBE (89 mM Tris, 89 mM boric acid, 1 mM EDTA) on a Sub-Cell electrophoresis unit (Bio-Rad, Richmond, Calif.). The gels were subsequently stained (1 h) with ethidium bromide (0.50 mg/ml), visualized with short-wave UV light, and photographed with a red filter.

REA pattern reproducibility was demonstrated by repeating the analysis at least five times. The similarities of isolate pairs were determined by visual comparison of the bands produced by dominant molecular mass fragments of greater than 6.6 kb. Similarity coefficients (S_{AB}s) were calculated by the method described by Schmid et al. (31). Band intensity was not considered in these calculations.

cDNA probe to 16S and 23S *Escherichia coli* rRNAs. Digoxigenin-labeled dUTP was incorporated into cDNA by the random oligonucleotide primed labeling method (Boehringer Mannheim, Indianapolis, Ind.). Alkaline phosphatase-treated rRNA (21 μg) and random hexanucleotide primer (50 pmol) were heated to 68°C for 5 min and then annealed by cooling to room temperature slowly. dATP, dCTP, and dGTP (50 nmol each), dTTP (32.5 nmol), digoxigenin-labeled dUTP (17.5 nmol), and avian myeloblastosis virus reverse transcriptase (500 U) were added, and the 550-μl reaction mixture was incubated at 42°C for 1 h. The digoxigenin-labeled probe was precipitated by the addition of LiCl (final concentration, 0.33 M) and 2 volumes of ethanol.

Southern hybridization. Gels of restriction-digested DNA prepared as described above were denatured in a solution of 1.5 M NaCl and 0.5 M NaOH for 1 h and were then neutralized in a solution of 1 M Tris and 1.5 M NaCl (pH 5.5) for 1 h. DNA was transferred to a nylon membrane (Boehringer Mannheim) in 10× SSC (1.5 M NaCl plus 0.15 M sodium citrate) by the method of Southern (34). DNA was fixed onto the membrane by baking at 80°C for 90 min. Membranes were incubated in prehybridization solution (2% Boehringer Mannheim blocking reagent, 5× SSC, 0.1% sarcosyl, 0.02% sodium dodecyl sulfate [SDS]) for 3 h at 68°C. The fixed DNA on the membrane was then hybridized with 1.4 μg of digoxigenin-labeled rRNA-cDNA probe in 15 ml of prehybridization solution overnight at 68°C. The membrane was next washed in a solution of 2× SSC–0.1% SDS for 10 min at room temperature; this was followed by two

TABLE 1. Comparison of *P. haemolytica* biotype T isolates cultured from eight bighorn sheep

Isolate no.	Animal	Collection date (mo-day-yr or PD)[a]	Sample type	Serotype	Beta hemolysis[b]	REA pattern[c]	Ribotype	Plasmid DNA
1	Lamb 1[d]	2-16-1989	Lung	3+, 4+, 10++	Yes	3	A	No
8	Ewe 8	4-11-1991	Oral	3+	No	1	B	No
9a	Ewe 9	4-11-1991	Oral	3+, 10++	Yes	10	A	Yes
9b	Ewe 9	4-11-1991	Oral	3+	No	11	B	No
10	Ewe 10	4-11-1991	Oral	4++	No	12	B	No
11	Ewe 11	4-11-1991	Oral	10++	Yes	13	A	No
12a	Ewe 12	5-18-1991	Oral	10++	Yes	7	A	No
12b	Ewe 12	4-11-1991	Oral	4+, 10++	Yes	3	A	No
18a	Lamb 18	PD 53	Nasal	3+, 10++	Yes	3	A	No
18b	Lamb 18	PD 55	ttw[e]	3+, 10++	Yes	3	A	No
18c	Lamb 18	PD 65	Oral	3+, 4+, 10++	Yes	3	A	No
18d	Lamb 18	PD 71	Oral	10++	Yes	3	A	No
17a	Lamb 17	PD 71	Nasal	3++, 10++	Yes	3	A	No
17b	Lamb 17	PD 71	Oral	3++, 10++	Yes	3	A	No
17c	Lamb 17	PD 73	ttw	3+, 4+, 10++	Yes	3	A	No

[a] Collection dates are recorded for older animals. Postcaesarian delivery dates (PD) are listed for lambs 17 and 18.
[b] Hemolytic activity was tested on Columbia blood agar with 5% sheep blood.
[c] Restriction enzyme analysis with *Hae*III.
[d] Lamb 1 was estimated to be 9 months old.
[e] ttw, transtracheal wash.

15-min washes in a solution of 0.1× SSC–0.1% SDS at 68°C. Labeled DNA was detected with anti-digoxigenin-labeled alkaline phosphatase (Boehringer Mannheim), according to the manufacturer's instructions. S_{AB}s were calculated as described above (31), except that dominant bands of all molecular masses were considered.

Plasmid analysis. Plasmid DNA was isolated by the alkaline lysis method (30) and was then analyzed on 1.0% agarose gels.

RESULTS

The two ewes (ewes 8 and 12) remained clinically normal prior to and following cesarean delivery of their lambs. Both lambs appeared to be free of respiratory disease through 7 weeks of age. However, signs of respiratory distress consistent with pneumonia developed in lambs 17 and 18 at 65 and 50 days PD, respectively (38).

A bacterium characteristic of a *Pasteurella* sp. was not detected in any of the nasal samples from the ewes, but both biotype T and 3 *P. haemolytica* were recovered from their tonsillar samples. *Pasteurella* sp. was not isolated from any of the lamb samples taken prior to 40 days PD. A biotype A *Pasteurella* sp. was isolated from the lambs' tonsillar samples on PD days 40 and 71 but not from samples collected from either lamb on PD day 53, when the predominant organisms were *P. haemolytica* biotype T. Biotype T *P. haemolytica* was isolated in pure culture, and high numbers were isolated from the lambs' transtracheal wash samples. The majority of *P. haemolytica* biotype T isolates from the ewes and lambs agglutinated in antiserum 10. Of these, several also agglutinated in either antiserum 3 or 4 or both antisera 3 and 4. The remaining isolates were either serotype 3 or 4 (Table 1).

A total of 25 isolates from ewes and 24 isolates from lambs were subjected to *Hae*III REA. Digested DNA showed distinct and reproducible differences in the 9 to 12 restriction enzyme fragments with molecular masses of greater than 6.6 kb. S_{AB} comparisons of the representative isolates from ewes (isolates 8, 9a, 9b, 10, 11, 12a, 12b) revealed S_{AB} values ranging from 0.09 to 0.95, indicating degrees of relatedness. These isolates were designated REA patterns 1, 10, 11, 12, 13, 7, and 3, respectively (Fig. 1; Table 1). S_{AB} values of ewe isolates 12b and lamb isolates 1, 17a to 17c, and 18a to 18d (REA pattern 3) were 1.0, indicating identity (Fig. 1; Table 1). The DNAs of 15 additional isolates collected from lambs 17 and 18 during the test period also produced REA pattern 3. No other biotype T REA patterns were isolated from lambs 17 and 18 throughout the study. REA patterns did not vary when the DNA isolated by ethanol precipitation was compared with that purified by CsCl density gradients.

Hybridization of *Hae*III-digested DNA to the digoxigenin-labeled cDNA *E. coli* 16S and 23S rRNA probe produced two patterns containing six dominant bands (Fig. 2). Ewe isolates 9a, 11, 12a, and 12b and lamb isolates 1, 17a to 17c, and 18a to 18d were not detectably different in their hybridization patterns (S_{AB} = 1.0) and were designated ribotype A. Probe hybridization to ewe isolates 8, 9b, and 10 also showed no detectable pattern differences (S_{AB} = 1.0) and were designated ribotype B. Comparisons between representative ribotype A and B isolates showed 50% homology of dominant band positions (S_{AB} = 0.5). All four REA patterns identified as ribotype A produced hemolysis on Columbia blood agar containing 5% sheep blood. All ribotype A isolates agglutinated strongly in antiserum 10 with various reactions in antisera 3 and/or 4 (Table 1). In contrast, ribotype B isolates were not hemolytic on this medium and agglutinated in antiserum 3 or 4 but not antiserum 10 (Table 1).

Low-molecular-mass (3- to 4-kb) plasmid DNA was present in isolate 9a. However, plasmid DNA was not observed in any of the other samples.

DISCUSSION

P. haemolytica isolates are common residents of the pharyngeal areas of clinically normal domestic lambs (2) and bighorn lambs (38, 41) as well as adult animals (37). Therefore, the presence of these organisms in the pharyngeal areas and nasal passages of the lambs at 40 days of age was not indicative of imminent respiratory disease. However, isolation of *P. haemolytica* in pure culture from the transtracheal samples of both lambs was conclusive evidence that this organism had invaded the lower respiratory tract and was at

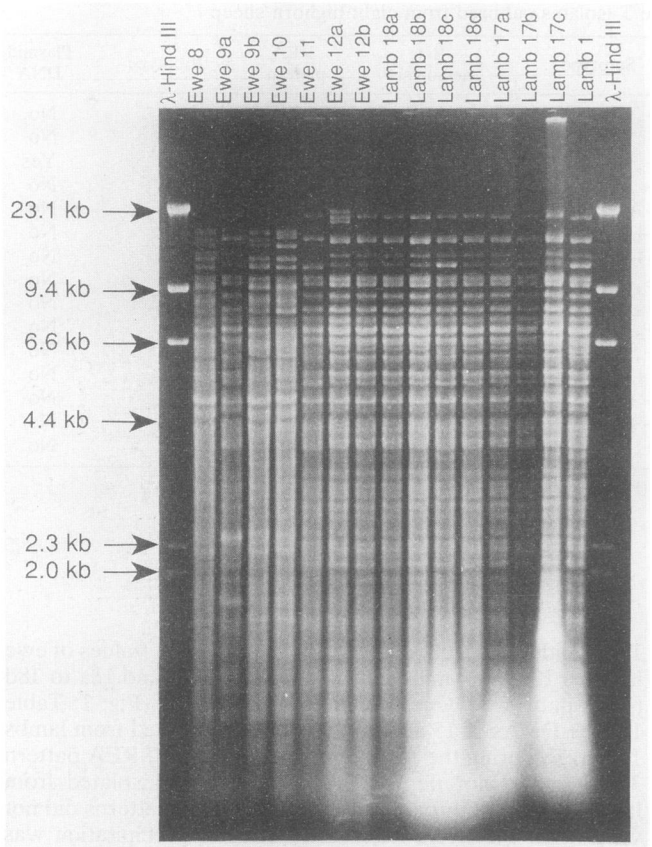

FIG. 1. Comparison of *Hae*III restriction profiles of *P. haemolytica* biotype T isolates from bighorn sheep. Ewe isolates are 8, 9, 9a, 9b, 10, 11, 12a, and 12b. Lamb isolates are 1, 17a to 17c, and 18a to 18d. Note the similarities of the REA patterns of ewe isolate 12b and all lamb isolates. Sizes are given in kilobases; λ *Hin*dIII restriction fragments serve as size standards.

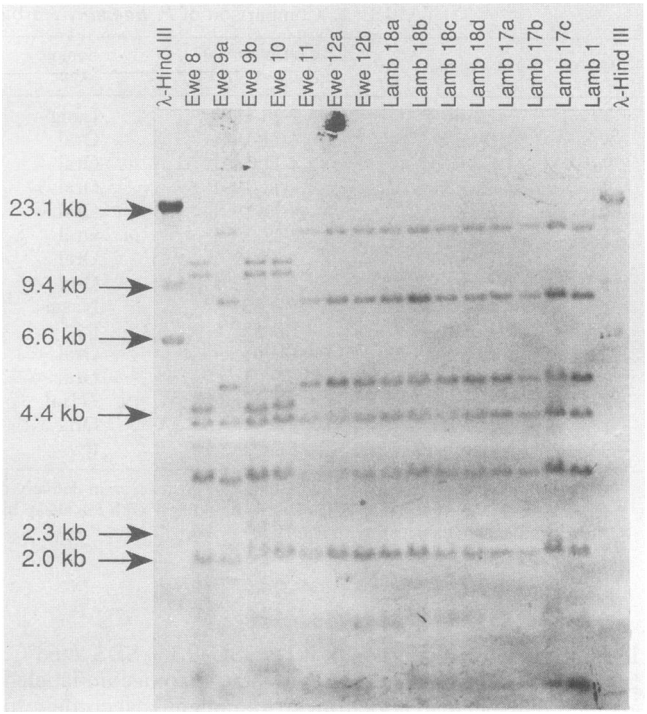

FIG. 2. Comparison of Southern hybridization blots of *Hae*III-digested *P. haemolytica* biotype T isolates from bighorn sheep, as listed in Fig. 1. Isolates 9a, 11, 12a, and 12b (from adult ewes) and 1, 17a to 17c and 18a to 18d (from lambs) are identified as ribotype A. Isolates 8, 9b, and 10 are identified as ribotype B.

least partially responsible for the signs of pneumonia. Pneumonia developed in the two cesarean-delivered lambs at an age comparable to that at which lambs in free-ranging herds were reported to die (1). Lambs in the free-ranging population which were observed to have signs of pneumonia died because of the disease or were killed by predators. Therefore, it was concluded that the cesarean-delivered lambs would not have survived this illness in the native habitat of their mothers. The biochemical and serological tests commonly used to identify *P. haemolytica* isolates do not have the discriminatory capacities necessary for epidemiological studies. Biochemically identical *P. haemolytica* biotype T isolates can show different REA patterns and ribotypes (Table 1) (33). The four recognized biotype T isolates frequently cross-react in typing sera (3). Therefore, serotyping of these organisms does not provide reliable data for use in epidemiological studies. In contrast, REA of chromosomal DNA can be used to identify and track the transmission of bacteria, as demonstrated in the present study. All *P. haemolytica* biotype T isolates from the transtracheal wash, nasal, and pharyngeal samples collected from the lambs had the same DNA REA pattern and ribotype as those of an isolate obtained from a lamb that was tested 2 years earlier and one isolate from the dam of a cesarean-delivered lamb. Isolation of the same REA pattern from the herd over a 2-year period may be indicative of the fact that this strain possesses unidentified virulence factors associated with an enhanced ability to colonize the upper respiratory tracts of bighorn sheep. A competitive colonization advantage has been associated with unique characteristics of a variety of bacterial pathogens (11, 32). Further characterization of *Pasteurella* isolates may reveal specific factors associated with variances in virulence and the natural host range.

The variance of REA patterns detected in samples from the ewes demonstrates that REA with *Hae*III can be used to distinguish among several isolates within a single biotype. In the present study, biochemically and serologically identical isolates from a single animal had unique REA patterns. The ability to discriminate among the related strains carried by these ewes demonstrates that REA with *Hae*III is a reliable tool for monitoring transmission of bacteria between animals or groups of animals. REA and ribotyping both provide data indicating ewe-to-lamb transmission of *P. haemolytica* in the present study. The fact that ribotyping correlated with beta-hemolytic activity and serotype in the present study is of interest. Additional isolates needs to be tested to further evaluate this correlation. Although ribotyping provides less discrimination between isolates than REA does, it can also be used to categorize and determine epidemiological relatedness among isolates.

ACKNOWLEDGMENTS

We gratefully acknowledge the efforts of the many individuals who assisted with capture operations to obtain pregnant bighorn ewes from herds with poor lamb survival. We also thank Jerry Zaugg and Michael Lane for the application of their surgical skills which made the first successful cesarean delivery of bighorn lambs a reality and to all who provided valuable advice and helped with the many hours of care required to raise the lambs.

REFERENCES

1. **Akenson, J. J., and H. A. Akenson.** Bighorn sheep herd composition and movements to lambing areas and summer ranges on Big Creek in Central Idaho. Bienn. Symp. North. Wild Sheep Goat Counc., in press.
2. **Al-Sulton, I. I., and I. D. Aitken.** 1985. The tonsillar carriage of *Pasteurella haemolytica* in lambs. J. Comp. Pathol. **95:**193–210.
3. **Biberstein, E. L.** 1964. Cross-reactions between types of *Pasteurella haemolytica*. Cornell Vet. **55:**495–499.
4. **Biberstein, E. L.** 1978. Biotyping and serotyping of *Pasteurella haemolytica*. Methods Microbiol. **10:**253–269.
5. **Biberstein, E. L.** 1979. The pasteurelloses, p. 485–514. *In* J. H. Steele (ed.), CRC handbook series in zoonoses. Section A. Bacterial, rickettsial and mycotic diseases. CRC Press, Inc., Boca Raton, Fla.
6. **Biberstein, E. L., M. Gills, and H. Knight.** 1960. Serological types of *P. haemolytica*. Cornell Vet. **50:**283–300.
7. **Biberstein, E. L., and D. A. Thompson.** 1966. Epidemiological studies on *Pasteurella haemolytica* in sheep. J. Comp. Pathol. **76:**83–94.
8. **Bisgaard, M., and R. Mutters.** 1986. Re-investigations of selected bovine and ovine strains previously classified as *Pasteurella haemolytica* and description of some new taxa within the *Pasteurella haemolytica*-complex. Acta Pathol. Microbiol. Immunol. Scand. **94:**185–193.
9. **Bradbury, W. C., A. D. Pearson, M. A. Marko, R. V. Congi, and J. L. Penner.** 1984. Investigation of a *Campylobacter jejuni* outbreak by serotyping and chromosomal restriction endonuclease analysis. J. Clin. Microbiol. **19:**342–346.
10. **Carter, G. R.** 1984. *Pasteurella*, p. 552–557. *In* J. G. Holt and J. R. Kreig (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
11. **Chandler, R. L., R. G. Bird, M. D. Smith, H. S. Anger, and B. A. Turfrey.** 1983. Scanning electron microscope studies on preparations of bovine cornea exposed to *Moraxella bovis*. J. Comp. Pathol. **93:**1–8.
12. **Cleary, P. P., E. L. Kaplan, and E. Livdahl.** 1988. DNA fingerprints of *Streptococcus pyogenes* are M type specific. J. Infect. Dis. **158:**1317–1323.
13. **Cox, N., J. Johnston, Z. Szarka, D. M. Wright, and L. C. Archard.** 1990. Characterization of an rRNA gene-specific cDNA probe: applications in bacterial identification. J. Gen. Microbiol. **136:**1639–1643.
14. **Davies, D. H., M. Herceg, B. A. H. Jones, and D. C. Thurley.** 1981. The pathogenesis of sequential infection with parainfluenza virus type 3 and *Pasteurella haemolytica* in sheep. Vet. Microbiol. **6:**173–182.
15. **Fox, M. L., R. G. Thomson, and S. E. Magwood.** 1971. *Pasteurella haemolytica* of cattle: serotype, production of beta-galactosidase and antibacterial sensitivity. Can. J. Comp. Med. **35:**313–317.
16. **Frank, G. H.** 1979. *Pasteurella haemolytica* and respiratory disease in cattle. Proc. U.S. Anim. Health Assoc. **83:**153–160.
17. **Frank, G. H.** 1982. Serotypes of *Pasteurella haemolytica* in sheep in the midwestern United States. Am. J. Vet. Res. **43:**2035–2037.
18. **Frank, G. H., and P. C. Smith.** 1983. Prevalence of *Pasteurella haemolytica* in transported calves. Am. J. Vet. Res. **44:**981–985.
19. **Frank, G. H., and G. E. Wessman.** 1978. Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. J. Clin. Microbiol. **7:**142–145.
20. **Gebhart, C. J., G. E. Ward, and M. P. Murtaugh.** 1989. Species-specific cloned DNA probes for the identification of *Campylobacter hyointestinalis*. J. Clin. Microbiol. **27:**2717–2723.
21. **Gilmour, N. J. L.** 1980. *Pasteurella haemolytica* infections in sheep. Vet. Q. **2:**191–198.
22. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur Microbiol. **137B:**165–175.
23. **Hoelein, A. B., S. P. Sazena, and M. E. Mansfield.** 1961. Studies on shipping fever of cattle. II. Prevalence of *Pasteurella* species in nasal secretions from normal calves and calves with shipping fever. Am. J. Vet. Res. **22:**470–472.
24. **Howard, C. J., and G. Taylor.** 1985. Immune responses to Mycoplasma infections of the respiratory tract. Vet. Immunol. Immunopathol. **10:**3–32.
25. **Jones, G. E., D. Buxton, and D. B. Harker.** 1979. Respiratory infections in housed sheep, with particular reference to mycoplasmas. Vet. Microbiol. **4:**47–59.
26. **Kilian, M., and W. Frederiksen.** 1981. Identification tables for the *Haemophilus-Pasteurella-Actinobacillus* group, p. 281–287. *In* M. Kilian, W. Frederiksen, and E. L. Biberstein (ed.), *Haemophilus, Pasteurella* and *Actinobacillus*. Academic Press, Inc., San Francisco.
27. **Kristiansen, B.-E., B. Sorensen, B. Bjorvatn, E. S. Falk, E. Fosse, K. Bryn, L. O. Frohlom, P. Gaustad, and K. Bovre.** 1986. An outbreak of group G meningococcal disease: tracing the causative strain of *Neisseria meningitidis* by DNA fingerprinting. J. Clin. Microbiol. **23:**764–767.
28. **Onderka, D. K., and W. D. Wishart.** 1984. A major bighorn sheep die-off from pneumonia in southern Alberta. Bienn. Symp. North Wild Sheep Goat Counc. **4:**356–363.
29. **Prince, D. V., and J. K. Clarke.** 1985. Serotypes of *Pasteurella haemolytica* from the respiratory tract of sheep in New Zealand. New Zealand Vet. J. **33:**76–77.
30. **Sambrook, J.** 1989. Plasmid vectors, p. 25–28. *In* J. Sambrook, E. F. Fritsch, and T. Maniatis (ed.), Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Schmid, J., E. Voss, and D. R. Soll.** 1990. Computer-assisted methods for assessing strain relatedness in *Candida albicans* by fingerprinting with the moderately repetitive sequence Ca3. J. Clin. Microbiol. **28:**1236–1243.
32. **Smith, G. R.** 1959. Isolation of two types of *Pasteurella haemolytica* from sheep. Nature (London) **183:**1132–1133.
33. **Snipes, K. P., R. W. Kasten, M. A. Wild, M. W. Miller, D. A. Jessup, R. L. Silflow, W. J. Foreyt, and T. E. Carpenter.** 1992. Using ribosomal RNA gene restriction patterns in distinguishing isolates of *Pasteurella haemolytica* from bighorn sheep (*Ovis canadensis*). J. Wildl. Dis. **28:**347–354.
34. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
35. **Stalheim, O. H. V.** 1990. Laboratory methods for mycoplasmas: animals, p. 583–584. *In* G. R. Carter and J. R. Cole, Jr. (ed.), Diagnostic procedures in veterinary bacteriology and mycology. Academic Press, Inc., San Diego, Calif.
36. **Thompson, D. A., J. Fraser, and N. J. L. Gilmour.** 1977. Serotypes of *Pasteurella haemolytica* in ovine pasteurellosis. Res. Vet. Sci. **22:**130–131.
37. **Ward, A. C. S., M. R. Dunbar, D. L. Hunter, R. H. Hillman, M. S. Bulgin, W. J. DeLong, and E. R. Silva.** 1990. Pasteurellaceae from bighorn and domestic sheep. Bienn. Symp. North. Wild Sheep Goat Counc. **7:**109–117.
38. **Ward, A. C. S., D. L. Hunter, M. D. Jaworski, V. M. Lane, J. L. Zaugg, and M. S. Bulgin.** Naturally occurring pneumonia in caesarian derived Rocky Mountain bighorn lambs. Bienn. Symp. North. Wild Sheep Goat Counc., in press.
39. **Ward, A. C. S., L. R. Stephens, B. J. Winslow, R. P. Gogolewski, D. C. Schaefer, S. D. Wasson, and B. L. Williams.** 1986. Isolation of *Haemophilus somnus*: a comparative study of selective media, p. 479–486. Am. Assoc. Vet. Lab. Diagnos., 29th Annu. Proc.
40. **Wesley, I. V., and J. H. Bryner.** 1989. Antigenic and restriction enzyme analysis of isolates of *Campylobacter fetus* subsp. *venerealis* recovered from persistently infected cattle. Am. J. Vet. Res. **50:**807–813.
41. **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. J. Wildl. Dis. **27:**53–60.
42. **Yates, W. D. G.** 1984. Interaction between viruses and bacteria in bovine respiratory disease. Can. Vet. J. **25:**37–41.