Bienn. Symp. North. Wild Sheep
and Goat Counc. 7:102-108.

SEROTYPES OF PASTEURELLA HAEMOLYTICA IN FREE RANGING ROCKY MOUNTAIN BIGHORN SHEEP

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Abstract: A total of 100 Rocky Mountain bighorn sheep (Ovis canadensis canadensis) from central Idaho, which had no known contact with domestic sheep, were monitored for causes of respiratory disease during Dec. 1988-Apr. 1989. Among 3 herds 2 were apparently free, or had a low incidence of respiratory disease, while the third herd had a high incidence of respiratory disease. Nasal and tonsillar samples from the sheep were cultured for Pasteurella haemolytica. Fifty-three isolates of P. haemolytica belonging to biotypes A (10), T (38) and 3 (5) were cultured from 38 animals. Six of the 10 biotype A isolates were untypable, 3 were identified as A_2 and 1 as A_{11}. Although some biotype T isolates agglutinated in individual antisera 3, 4 or 10 and were therefore identified as serotypes T_3 (5), T_4 (2) or T_{10} (5) respectively, most isolates were atypical in that they agglutinated in 2 or 3 of the antisera. Seventy-two percent of the isolates were hemolytic on blood agar compared to a low incidence of hemolytic isolates reported in other studies.

Pasteurella species have been incriminated as important pathogens of bighorn sheep, causing pneumonia and often death (Marsh 1938, Post 1962, Foreyt and Jessup 1982, Spraker et al. 1984). Naturally occurring outbreaks of pneumonia caused by Pasteurella haemolytica have been reported in free-ranging bighorns (Taylor 1976, Wishart et al. 1980, Spraker and Hibler 1982, Onderka and Wishart 1984). Some outbreaks of pneumonia have been suspected of being caused by contact with domestic sheep. Onderka and Wishart (1988) and Foreyt (1989) suggest that certain types of P. haemolytica may be common to domestic sheep and that some of these types may cause fatal pneumonia in bighorn sheep. A few investigators (Taylor 1976, Onderka et al. 1988, Onderka and Wishart 1988) have used serotyping of P. haemolytica to investigate pneumonia in bighorn sheep. However, little information exists concerning specific serotypes in healthy or diseased bighorn sheep.

Pasteurella haemolytica isolates are tested for biochemical reactions to establish biotypes and with antisera to detect specific antigens on the organisms for serotyping. Biotyping and serotyping of

P. haemolytica are commonly performed in investigating pneumonia in domestic livestock (Biberstein and Thompson 1966, Thompson et al. 1977, Gilmour 1980, Fraser et al. 1982, Confer et al. 1988). The P. haemolytica group currently has 3 recognized biotypes, A, T and 3 (Kilian and Frederiksen 1981). Serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13 and 14 can be distinguished in A biotype while the T biotype has 4 recognized serotypes; 3, 4, 10 and 15. Serologically untypable strains, with biochemical characteristics typical of biotypes A and T also occur. Serotype designations have not been developed for biotype 3 organisms. Although hemolysis is a cardinal characteristic of P. haemolytica, non-hemolytic strains have been reported. Isolates from domestic sheep are characteristically hemolytic, whereas a predominance of non-hemolytic strains has been reported in bighorn sheep (Onderka and Wishart 1988).

Serotyping increases precision in detecting organisms associated with disease, following the course of an epizootic, and detecting transmission among animal populations. In this study, serotyping of P. haemolytica from healthy and diseased bighorn sheep, having no known contact with domestic sheep, was conducted to establish whether particular serotypes were indigenous in a given bighorn sheep and which were associated with disease.

We acknowledge the assistance of the Idaho Department of Fish and Game, Idaho State Department of Agriculture, and The Wilderness Research Center of the University of Idaho.

STUDY AREA

The study was conducted in central Idaho along the main Salmon River and its tributaries. Major tributaries included Morgan Creek, near Challis; Panther Creek, near Shoup; Middle Fork of the Salmon River and Big Creek, both within the Frank Church River of No Return Wilderness.

The topography is characterized by high mountain peaks and ridges dissected by numerous deep, narrow valleys. Elevations range from 600-2800 m. The main study area was on sheep winter ranges at lower elevations. The Salmon River and Middle Fork canyons cut deep into the huge batholith, a vast granitic mass measuring 112 by 385 km. The Salmon River canyon is one of the most rugged in North America and is hot and dry in summer. Annual precipitation in the study areas ranges between 23-38 cm. During winter, southern exposures often are bare, while there are 45-65 cm of snow on north-facing slopes.

Ponderosa pine (Pinus ponderosa) occupies areas along lower river breaks. In other areas, grassy slopes reach to the river. The principal grass is bluebunch wheatgrass (Agropyron spicatum) and important shrubs are curlleaf mountain mahogany (Cercocarpus ledifolius), bitterbrush (Purshia tridentata) and big sagebrush (Artemisia tridentata).

METHODS

One hundred free-ranging Rocky Mountain bighorn sheep consisting of

71 adult females, 21 adult males, (1½-11 years of age), and 8 lambs (7-11 months), were either captured (87), killed (7), found dead (5) or died during capture (1) in the Salmon River drainage during Dec. 1988-Apr. 1989. Sheep were sampled from 3 populations within the drainage. Sixty were sampled from Morgan Creek near Challis. This herd consists of about 250 animals and has been increasing over the last 5-6 years. Thirty sheep were sampled from the main Salmon River herd, below the town of Shoup. This herd consists of about 485 animals and has been increasing recently, however not to the extent of the Morgan Creek herd. The Big Creek herd, in a tributary of the Middlefork of the Salmon River consists of about 200 animals, of which 10 were sampled. This herd has experienced low lamb production for the past 3-4 years but population is above 10-year average.

Most sheep were captured using a net gun (Coda Enterprises, Inc., Mesa, Arizona, 59 sheep) fired from a helicopter or using a projectile dart (Cap-Chur gun, Palmer Chemical and Equipment Co., Ltd., Douglesville, Georgia, 29 sheep) containing either etorpine hydrochloride (American Cyanamid Co., Princeton, New Jersey) or carfentanil citrate (Wildlife Laboratories, Inc., Fort Collins, Colorado). Diprenorphine (American Cyanamid Co.) was administered by hand-held syringe to reverse both drugs. Animals were aged by tooth eruption and horn development. All live-captured animals were examined by a veterinarian for signs of illness and, except for 2 adult ewes, released after samples were collected.

Swab samples were taken from nasal passages of all sheep using rayon-tipped, 20 cm, swabs (Culturette, Marion Laboratories Inc., Kansas City, Missouri). Tonsil biopsies were obtained from bighorns as described by Dunbar et al. (1990), and were transported in modified Stuart's transport medium (Culturette, Marion Laboratories Inc.) to the laboratory. Two apparently healthy adult ewes were transported to the Caine Veterinary Teaching and Research Center, Caldwell, Idaho, where they were euthanized and necropsied. Eight necropsies were performed in the field.

Tissue samples from field necropsies were individually placed in sterile plastic bags (Whirl-Pac Bags, Nasco, Modesto, California), iced and transported by express mail to the University of Idaho, Caine Veterinary Teaching and Research Center, Caldwell, Idaho or the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, Washington. Samples were cultured within 48-72 hours from collection.

Surfaces of sufficiently large tissue samples were seared to destroy surface bacteria, prior to culture. Sterile instruments were used to obtain tissues for culture from beneath seared areas. Tonsillar tissues of $\geq$ 1g were placed in sterile plastic bags with sterile Brain-Heart-Infusion (Becton Dickinson Microbiology Systems, Cockeysville, Maryland) broth and macerated in a tissue homogenizer (Tekmar Co., Cincinnati, Ohio) to release bacteria from crypts. Smaller tonsil samples were rubbed on culture media to enhance bacterial release.

All samples were cultured on Columbia Agar (Becton Dickinson

Microbiology Systems) with 5% citrated sheep blood and on a selective Columbia Blood Agar with added vancomycin (6ug/ml) and nystatin (12.5ug/ml) (Sigma Chemical Co., St. Louis, Missouri), a medium which is selective for Pasteurellaceae. The inoculated culture media were incubated at 37C and examined daily for at least 3 days.

Bacterial isolates which were gram-negative, oxidase-positive and produced acid reactions when inoculated into triple-sugar-iron (Becton Dickinson Microbiology Systems) agar slants were evaluated by additional biochemical tests to determine if they were Pasteurella spp. Biotypes and serotypes were determined according to published procedures (Frank and Wessman 1978, Kilian and Frederiksen 1981).

RESULTS

Sixty sheep from the Morgan Creek herd had a high prevalance of mild to severe signs of respiratory disease with coughing, muco-purulent nasal discharge and abnormal lung sounds unon auscultation. Several animals in addition to those captured, were observed coughing and in poor health. Thirty sheep from the Salmon River herd included a moderate number with signs of respiratory disease, ie. coughing and clear nasal discharge. However, 1 adult ram had severe respiratory disease. Ten sheep from the Big Creek herd showed no signs of respiratory disease and others observed from the ground were all apparently healthy.

Pasteurella haemolytica was found in 32% of the sheep from Morgan Creek, 50% from Salmon River and 30% from Big Creek. Fifty-three isolates of P. haemolytica were cultured from 38 of 100 bighorn sheep and identified as biotypes, A (10), T (38) and 3 (5). Three of the 10 biotype A isolates reacted in A_2 antisera, 1 in A_{11} and the remaining 6 were untypable with available typing sera. Five of the 38 biotype T isolates were classified as T_3, 2 as T_4 and 5 as T_{10}. The remainder of the biotype T isolates reacted in more than 1 of the type sera in combinations of T_3, T_4, T_{10} (N=21), T_3, T_4 (N=1) and T_4, T_{10} (N=4).

Biotype A was isolated only from nasal passages, except for 1 isolate from the larynx of an adult ewe that was euthanized and necropsied. The A biotypes were isolated at a greater frequency from lambs (43%) than from adult bighorns (18%) from the Morgan Creek and Salmon River herds. No lambs were sampled from the Big Creek herd.

Pasteurella haemolytica biotype T was isolated from both tonsils and nasal passages of bighorn sheep but at a higher rate from nasal passages of sheep from herds showing signs of respiratory disease (Morgan Creek 68%, Salmon River 13%) than from the apparently healthy Big Creek herd (0%).

Three of the 5 isolates of biotype 3 were from tonsillar tissue and 2 from nasal passages. No correlation was observed between serotypes of P. haemolytica and either sex or age, except as previously mentioned for lambs.

Biotypes A, T and 3 were isolated at approximately the same

frequency from the Salmon River and Morgan Creek herds. Neither biotype A nor 3 was isolated from the Big Creek herd. Pasteurella haemolytica was isolated from 3 tonsils but not from nasal swabs of sheep in the Big Creek herd. Serotypes T_{10} (hemolytic) and T_4, T_{10} (hemolytic) combination were the only isolates from the Big Creek herd.

Thirty-eight of the 53 isolates (72%) were hemolytic on blood agar. Twenty-eight of the 100 sheep had at least 1 isolate of P. haemolytica that was hemolytic. Eighty-four percent of the isolates from the Morgan Creek herd, 56% from the Salmon River herd, and 100% from the Big Creek herd, were hemolytic. Both hemolytic and non-hemolytic isolates of of biotypes A, T and 3 were found.

DISCUSSION

Biotyping and serotyping P. haemolytica has enhanced understanding of this organism and associated disease and development of control measures for preventing P. haemolytica pneumonia in domestic animals. However, biotyping and serotyping P. haemolytica in bighorn sheep have only recently occurred (Taylor 1976, Onderka et al. 1988, Foreyt 1989, Dunbar et al. 1990) and investigations have been limited. Study of suspected transfer of P. haemolytica from domestic sheep to bighorn sheep has been hampered by lack of knowledge about the carrier states of sheep for specific serotypes of P. haemolytica by domestic sheep and bighorn sheep before they commingled. The carrier states of animals in these studies were based on isolation of Pasteurella from nasal samples. However, we have found that tonsillar biopsy samples are more reliable than nasal samples for detecting Pasteurella carriage in bighorn sheep (Dunbar et al. 1990). The use of tonsil biopsies or tonsillar swabs and serotyping would identify carrier animals and possibly allow tracking the transfer of P. haemolytica between species or individuals.

Biotypes and serotypes of P. haemolytica found in this survey include several not previously described. Taylor (1976) found serotype T_3 in bighorns (O. c. nelsoni) from Nevada. Onderka et al. (1988) reported serotypes T_3, T_4 and T_{15} in wild bighorns from Alberta, Canada, and Foreyt (1989) reported serotypes T_3, A_2, A_9 and A_{11} in a captive bighorn sheep herd, in Washington state, after they commingled with domestic sheep. No isolations of P. haemolytica were found in nasal samples from the bighorn sheep prior to the introduction of domestic sheep in his study, and no tonsillar sampling was done prior to commingling. In our study, serotypes T_3, T_4, T_{10}, A_2, A_{11} and biotype 3 were isolated from both nasal and tonsil samples. We also found that the majority of the biotype T isolates of P. haemolytica agglutinated in 2 or 3 of the antisera. The finding of biotype T isolates which reacted > 1 serotype is rare in domestic species. These may represent organisms unique to the bighorns.

Onderka and Wishart (1988) used hemolytic serotype T_{10} to trace the transfer of P. haemolytica from domestic sheep to captive bighorn sheep, since T_{10} was not isolated from nasal passages of bighorn sheep prior to commingling with domestic sheep infected with hemolytic T_{10}. However, in this study, hemolytic T_{10} was commonly carried in the palatine tonsils of free-ranging bighorn sheep having no known contact

with domestic sheep. We observed that most (72%) isolates of P. haemolytica, from bighorn sheep were hemolytic, in contrast to 7% in Alberta bighorns (Onderka and Wishart 1988).

Onderka et al. (1984) observed hemolysis characterized most P. haemolytica isolates from domestic sheep and non-hemolysis characterized isolates from bighorn sheep in Alberta. Their study suggested that if bighorn sheep carried hemolytic P. haemolytica, these isolates may have been transferred from domestic sheep. The bighorns in our study carried mainly hemolytic isolates of P. haemolytica despite no known contact with domestic sheep. However, we cannot exclude that the hemolytic isolates of P. haemolytica in our bighorn sheep did not historically originate from domestic sheep.

In this study, no correlation was found between serotypes of P. haemolytica and signs or severity of respiratory disease. However in individual animals with signs of respiratory disease, as was the case in the Morgan Creek herd, the T biotypes were isolated at a higher frequency from nasal passages compared to sheep from the 2 herds with less or no signs of respiratory disease. Consequently, it appears that the T biotype, normally carried in the palatine tonsils of most apparently healthy sheep, proliferate as a sheep becomes stressed, and invade nasal passages, where biotype A is a more common commensal.

The roles of serotypes and of the characteristic of hemolysis of P. haemolytica in the bighorn sheep pneumonia-complex needs further investigation. Techniques such as fingerprinting based on bacterial proteins and DNA components, will help to characterize P. haemolytica strains that vary in frequency among animal species and may have greater virulence for some species. These techniques will help to detect the spread of bacterial pathogens among herds and species. However, until these new techniques are developed and tested, serotyping will continue to be an important tool.

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