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ISOLATION OF *PASTEURELLA HAEMOLYTICA* FROM TONSILLAR BIOPSIES OF ROCKY MOUNTAIN BIGHORN SHEEP

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ABSTRACT: Isolations of *Pasteurella haemolytica* were compared from tonsillar biopsies versus nasal passages for 29 free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) from central Idaho. Overall, *P. haemolytica* was isolated from 11 (38%) of 29 sheep. Two (18%) of the 11 positive samples were from only nasal passages compared to eight (73%) from tonsillar biopsies. *Pasteurella haemolytica* biotype T was isolated from tonsils of nine sheep and from nasal biopsies. *Pasteurella haemlytica* biotype T was isolated from tonsils of nine sheep and from nasal passages of only one sheep. Two sheep were positive for *P. haemolytica* biotype A from nasal passages. Culturing tonsillar biopsies as compared to nasal swab samples was a more reliable technique in detecting *P. haemolytica*, especially biotype T, in bighorn sheep.

Key words: *Pasteurella haemolytica*, pneumonia, Rocky Mountain bighorn sheep, *Ovis canadensis*, *canadensis*, tonsillar biopsies, prevalence, survey.

INTRODUCTION

Pasteurella haemolytica has been incriminated as an important pathogen in the respiratory disease complex of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) (Spraker and Hibler, 1982; Onderka and Wishart, 1988; Foreyt, 1989). Although other bacteria, viruses and parasites also may be involved (Post, 1962; Forrester, 1967; Woolf et al., 1970; Dunbar et al., 1985), *Pasteurella sp.* may be the only infectious agent isolated in some outbreaks of pneumonia (Howe, 1966; Onderka and Wishart, 1984). Recent studies by Foreyt (1989) indicate that the T biotype of *P. haemolytica* may be a more important pathogen in bighorn sheep than the A biotype. He also postulates that biotype T, known to be carried by normal healthy domestic sheep (Biberstein and Thompson, 1966), is transmitted to bighorns from domestic sheep resulting in severe pneumonia and often death. This suggestion is based on cultures of nasal swab samples of bighorn sheep from which *P. haemolytica* were not isolated prior to introduction of domestic sheep. Requisite to such conclusions would be an assumption that bighorn sheep did not carry *P. haemolytica*, especially the suspected pathogen, biotype T, prior to the introduction of domestic sheep.

Customarily, nasal passages of live bighorn sheep have been swabbed and cultured for *P. haemolytica*. However, isolation of *P. haemolytica*, particularly biotype T, from nasal passages of live healthy animals has had mixed results (Gilmour et al., 1974; Frank, 1982; Onderka and Wishart, 1984). Gilmour et al. (1974) reported that the T biotype of *P. haemolytica* were isolated at a greater rate from tonsillar tissue than from the nasopharynx of dead domestic sheep. It appears then, that if tonsillar tissue of live sheep were cultured for *P. haemolytica*, more accurate results might be derived.

This study describes methods of obtaining tonsillar biopsies and results of culturing tonsillar biopsies and nasal swabs of live bighorn sheep. This was part of an investigation to determine the health of a herd of bighorn sheep suspected of having an epizootic of pneumonia.

MATERIALS AND METHODS

Twenty-nine free-ranging Rocky Mountain bighorn sheep consisting of 25 adult females, three adult males, and one lamb were captured

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in the Salmon River drainage of central Idaho, USA; (45°05′ To 45°20′N, 114°20′ To 114°58′W) between December 1988 through March 1989 using a net gun (n = 10) (Coda Enterprises, Inc., Mesa, Arizona 85203, USA) fired from a helicopter or using a projectile dart (Cap-Chur gun, Palmer Chemical and Equipment Co. Ltd., Douglasville, Georgia 30134, USA) containing either etorpine hydrochloride (n = 9) (American Cyanamid Co., Princeton, New Jersey 08540, USA) or carfentanil citrate (n = 10) (Wildlife Laboratories, Inc., Fort Collins, Colorado 80524, USA). Diprenorphine (American Cyanamid Co., Princeton, New Jersey 08504, USA) was administered by hand-held syringe to reverse the actions of both drugs. All animals were released after samples were collected. Samples were individually identified by numbering.

Swab samples for microbiologic examination were taken from nasal passages of live sheep using rayon tipped, 20 cm, swabs wetted with modified Stuart's transport medium (Culturette, Marion Laboratories, Inc., Kansas City, Missouri 64114, USA).

Tonsil biopsies were obtained from bighorns by holding the jaws open, approximately 7 cm, with a speculum which allowed adequate viewing but only minimal discomfort to the animal, and retracting the tongue with a tongue forceps. A laryngoscope with a light source was used to depress the tongue base and permit lighted viewing of the tonsillar sinus. An 18 cm curved vascular hemostat was used to obtain a pinch biopsy, approximately 1 mm × 2 mm × 1 mm, from the palatine tonsil. The tonsillar tissue was placed in modified Stuart's transport medium.

Samples were placed on ice and transported by express mail to the laboratory (University of Idaho, Caine Veterinary Teaching and Research Center, Caldwell, Idaho 83605, USA) for culturing, within 48 to 72 hr from time of collection. All samples were cultured on Columbia Blood Agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030, USA) with 5% citrated sheep blood (CBA) with added vancomycin (6μg/ml) and Nystatin (12.5 μg/ml) (Sigma Chemical Co., St. Louis, Missouri 63178, USA), a medium which has been found to be selective for members of the Pasteurellacae. The inoculated culture media were incubated at 37 C and examined daily for 3 days.

All bacterial colony types were screened to identify *Pasteurella* spp. Bacterial isolates which were gram-negative, oxidase positive and produced acid reactions when inoculated into triple-sugar-iron (TSI) (Becton Dickinson Microbiology Systems) agar slants were further evaluated to determine species and biotypes according to published procedures (Kilian and Frederiksen, 1981).

RESULTS

Twenty-seven of 29 bighorn sheep sampled were apparently healthy and in good body condition. Only two bighorns, one adult ewe (#4) and one adult ram (#54), had signs of mild to severe respiratory disease with mucopurulent nasal discharge and were coughing. In addition, the adult ram (#54) was emaciated and had abnormal lung sounds upon ausculation. Consequently, the suspected epizootic of pneumonia in this herd was not occuring at the time of this study.

Pasteurella haemolytica was isolated from 11 (38%) of 29 bighorn sheep. Eight (73%) of the 11 positive samples were obtained from tonsils only, two (18%) were obtained from nasal passages alone (#4 and #49) and one (9%) was obtained from both nasal passages and tonsils of an apparently healthy lamb(#8). One of the 11 sheep, an apparently healthy ram (#53), was poitive for *P. multocida* from the nasal passage and for *P. haemolytica* from the tonsils. *Pasteurella haemolytica* biotype T was isolated from only tonsillar tissue in nine animals and from the nasal pasage of the sick ewe (#4). The sick ram (#54) was positive for *P. haemolytica* biotype T from the tonsils only. *Pasteurella haemolytica* biotype A was isolated from the nasal passages from two of the 11 sheep, an apparently healthy lamb (#8) and ewe (#49).

DISCUSSION

In recent studies of domestic and bighorn sheep interaction (Onderka and Wishart, 1988; Foreyt, 1989), investigators have used nasal swabs for sampling bacterial pathogens, primarily *P. haemolytica*, from clinically normal domestic and bighorn sheep. However, after commingling, fatal acute pneumonia occurred in the bighorn sheep. The domestic sheep were suspected of transmitting the primary pathogen because *P. haemolytica* was isolated from nasal swabs of several domestic but no bighorn sheep before con-

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tact, and at necropsy from the lungs of two bighorn sheep that died from pneumonia.

Earlier studies in both domestic sheep (Gilmour et al., 1974; Al-Sultan and Aiken, 1985) and bighorn sheep (Onderka et al., 1988) indicate that *P. haemolytica* is often carried in the tonsils of healthy sheep. Our work substantiates this in bighorns. Consequently, swab samples from only nasal passages, especially clinically normal bighorn sheep, may be expected to yield a much lower percentage of positive samples as compared to culturing tonsils for *P. haemolytica*, especially biotype T.

Onderka and Wishart (1988) cultured cut sections of tonsils from hunter-killed bighorns and found that 25% of 61 clinically normal bighorn sheep in Alberta carried *P. haemolytica*, mostly biotype T, whereas swab samples from nasal passages of 239 clinically normal bighorns were negative (Onderka and Wishart, 1984). In this study, only 10% (3 of 29) of nasal swabs from bighorn sheep were positive for *P. haemolytica* compared to 31% (9 of 29) from tonsillar biopsies alone. Gilmour et al. (1974) reported *P. haemolytica*, biotype T, was isolated from tonsils of clinically normal domestic sheep 15 times more often than from nasal passages. In this study of bighorn sheep, we isolated *P. haemolytica* biotype T nine times more often from tonsils versus nasal passages. Therefore, if tonsillar tissue from live or dead bighorn and domestic sheep were cultured for *P. haemolytica*, more accurate information regarding carrier state could be obtained.

Unfortunately, it is more difficult to biopsy tonsillar tissue in ruminants than in other animals. The palatine tonsils of domestic ruminants and bighorn sheep are located on the lateral wall of the oropharynx, near the attachment of the soft palate and within the tonsillar sinuses. Palatine tonsils are composed of numerous follicles arranged around a central sinus into which crypts open. In ruminants, tonsillar sinuses are surrounded by tonsil; tonsillar fossae surround the tonsils in other species (Sisson and Grossmon, 1975). Consequently, difficulty in viewing the area and the long distance to the tonsillar sinuses make biopsy more difficult with the live animal. However, with adequate instrumentation and experience, biopsy can be accomplished in the field.

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BOOK REVIEW . . .

Symposium on Fish Vaccination, O.I.E. Fish Disease Commission, 12, rue de Prony 75017, Paris, France. 1988. 248 pp.

The book reporting on and entitled "Symposium on Fish Vaccination" is a compilation of topics discussing the theoretical background and practical results on the immunization of fish against infectious diseases." The symposium was held in February 1984 and the report was reprinted in November 1988. The symposium was to serve as a vehicle to introduce and involve veterinarians in fish health and maintenance. Although many aspects of the symposium are relevant in 1989, the vast majority of the topics desperately need updating. The overall quality of the report could be enhanced greatly by the inclusion of addenda to update chapters where possible. For example, the chapter dealing with "Immunization against viral diseases occurring in cold water" is a fairly detailed discussion of IPN, SVC, VHS, and IHN virus infectious, even to the level of describing the architecture of the virus and the various mechanisms of virulence. However, the following chapter "Immunization of warm water fish against five important pathogens" fails to describe the simplest of facts about the pathogens being discussed, such as gram stain reaction and cell shape or size. Although commercial warm water fish farming is a development of the past 20 yr and limited research data was available before 1984, some effort should have been employed to present similar details in all chapters and update chapters where possible. The pictures and photomicrographs of diseased fish and histopathology are severely lacking in quality and should not have been included in the present form. Aside from the previous noted deficiencies, the report does serve as an excellent primer for veterinarians and students pursuing studies in fish disease/pathology to introduce them to a limited number of fish species and pathogens.

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