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ISOLATION OF *PASTEURELLA* SPP. FROM FREE-RANGING AMERICAN BISON (*BISON BISON*)

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ABSTRACT: From November 1991 through March 1992, nasal and pharyngeal swab samples were collected from 45 bison (*Bison bison*) from Yellowstone National Park, Montana (USA) and cultured for *Pasteurella* spp. Thirteen isolates of *Pasteurella* spp. were recovered from 10 (22%) of the animals. Ten isolates were from pharyngeal samples in contrast to three isolates from nasal samples. *Pasteurella haemolytica* (six biotype T, two biotype A, and two biotype 3) was the predominant *Pasteurella* species. Five biotype T isolates were serotype 4 and the sixth agglutinated in antisera 3, 4, and 10. Both biotype A isolates were untypable with antisera to recognized type strains. *Pasteurella multocida* was isolated from the pharyngeal samples of one animal. Two isolates could not be identified to species.

Key words: *Pasteurella* spp., bison, *Bison bison*, Yellowstone National Park.

INTRODUCTION

The population of American bison (*Bison bison*) in Yellowstone National Park, Montana (USA) is estimated to consist of approximately 3,000 animals (Breining, 1992). Pasteurellosis in domestic livestock commonly is associated with *Pasteurella haemolytica* or *Pasteurella multocida,* and a variety of respiratory viruses and stress factors (Shoo, 1989).

Pasteurella species are common commensals in the upper respiratory tract of domestic livestock, wild mammals, and avian species (Rosen, 1971). *Pasteurella* spp. have been incriminated as the cause of disease in wild ruminants (Thorne, 1982) including hemorrhagic septicemia in American bison (Heddleston et al., 1967). Hemorrhagic septicemia due to infection with *Pasteurella multocida* B-2 or E-2 strains can cause high mortality in infected domestic cattle (Francis et al., 1980). These strains may no longer be present in the domestic cattle population in the United States (Carter, 1982). It is currently unknown if American bison are a reservoir for the B-2 strain. The prevalence of *Pasteurella* spp. in Yellowstone bison is also unknown. Our objective was to determine the prevalence and species of *Pasteurella* spp. present in nasal and pharyngeal swab samples of apparently healthy animals.

METHODS AND MATERIALS

During the study period, winter movement of bison from the Northern herd, at Yellowstone National Park into Gardiner, Montana (45°3′N, 110°53′W) began in November 1991 and continued through March 1992. Animals which remained outside the Park's boundaries after attempts were made to haze them back into the Park, were killed by rifle shot in compliance with the Montana State Department of Livestock and Department of Fish, Wildlife, and Park's Order of Destruction Number B-2. Nasal swab samples from 45 animals were collected using a rayon-tipped swab system with a Amies modified transport medium containing charcoal (Precision Dynamics Corporation, San Fernando, California, USA). Pharyngeal samples were collected from all animals with Accu-CulShur™ (Accu-Med Corporation, Pleasantville, New York, USA) swabs which provided a sterile protective sleeve for collection of samples from deep cavities. Samples were shipped on ice and received at the University of Idaho, Caine Veterinary Teaching and Research Center 48 to 96 hr after collection.

All samples were inoculated onto a non-se-

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lective Columbia blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) containing 5% ovine blood (CBA) and Columbia blood agar with 10% bovine blood plus antibiotics (CBAA), selective for *Pasteurella* spp., as described by Jaworski et al. (1993). Plates were incubated at 35 C in an atmosphere with 10% added CO_2 and bacterial growth was evaluated daily for 3 days. Bacterial colonies which resembled *Pasteurella* spp. were identified and assigned to a biotype by the procedures of Kilian and Frederiksen (1981). Isolates identified as *Pasteurella haemolytica* were serotyped with antisera obtained from the National Animal Disease Center (Ames, Iowa, USA) using the slide agglutination procedure of Frank and Wessman (1978).

RESULTS

Most samples collected from the bison were contaminated with rumen contents and dirt. This contamination occurred due to the impact of falling to the ground upon fatal gun shot. Colonies characteristic of *Pasteurella* spp. were detected on CBAA medium inoculated with samples from 10 animals and from one sample inoculated on the CBA medium. *Pasteurella* spp. were isolated from 10 (22%) of 45 animals tested. Ten of 13 isolations were from pharyngeal swabs. Two biochemically distinct *Pasteurella* spp. were isolated from each of two different animals. Two biochemically different isolates were isolated from the pharyngeal sample of one animal and different *Pasteurella* spp. biotypes were isolated from nasal and pharyngeal samples of another animal. Ten of the 13 isolates were identified as *Pasteurella haemolytica*: six biotype T, two biotype A, and two biotype 3. Five biotype T isolates were identified as serotype 4 and one agglutinated in antisera for serotypes 3, 4, and 10. Neither biotype A isolates agglutinated in any of the identified antisera for that biotype. The species of two *Pasteurella* spp. could not be determined biochemically. A single isolate of *Pasteurella multocida* was detected on CBA only.

DISCUSSION

Numerous organisms, including *Pasteurella* spp., *Actinomyces* spp., *Acinetobacter* spp., *Neisseria* spp., *Moraxella* spp., *Staphylococcus* spp., and *Streptococcus* spp. are commonly isolated from nasal mucosa and the pharyngeal area of ruminants (Carter and Cole, 1990). Most of these organisms are believed to have a predilection for these sites and are capable of colonizing the upper respiratory tract. Some organisms isolated from bison in this study may be incidental to inhalation of dust, licking habits, and forage ingestion. The first five genera listed above exist as obligate commensals but may survive short periods of time outside of an animal host. *Staphylococcus* spp. are common on the skin of animals and may occur in nasal or pharyngeal samples due to smelling and licking activities (Kloos and Schleifer, 1981). Some *Streptococcus* spp. are common in nasal and oral cavities but others are common on the skin of animals and on the surface of plants (Facklam and Wilkinson, 1981).

The presence of rumen contents and dirt in the nasal passages and pharyngeal area were unavoidable factors associated with the lethal shot to the head. This contamination may have altered the types and numbers of bacteria that would have been present in the samples. Consequently, no attempts were made to identify normal bacterial flora, with the exception of *Pasteurella* spp. Since *Pasteurella* spp. are obligate commensals, isolation of these organisms is considered a true reflection of a carrier state.

Pasteurella spp. are moderately fastidious bacteria which grow readily on standard non-selective media containing blood or serum. The prevalence of *Pasteurella* spp. carriers in the Yellowstone National Park bison population appears to be low in relation to the prevalence reported in other species (Hoerlein et al., 1961; Gilmour et al., 1974; Al-Sultan and Aitken, 1985; Ward et al., 1990). Various factors such as sampling site and sample handling could have contributed to artificial lowering of the estimated prevalence of the organism among the bison (Wild and Miller, 1991). These may have included loss of

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Pasteurella spp. viability due to contamination of samples with rumen contents or dirt, loss of viability on swabs during transit to the Caine Center, and inhibition due to overgrowth by other bacteria.

Isolation of *Pasteurella* spp. from this group of Yellowstone bison was not associated with evidence of disease in any of the animals. In 1922, an epizootic of pasteurellosis occurred in Yellowstone National Park bison (Gochenour, 1924); however, there have been no reports of pasteurellosis in the Park's bison since that time. In 1986 and 1987, systemic pasteurellosis caused by *Pasteurella multocida* was attributed to the deaths of 48 elk (*Cervus elaphus*) (Franson and Smith, 1988). This epizootic occurred on the National Elk Refuge near Jackson, Wyoming (USA). Due to the moments of both the elk and bison that inhabit the Greater Yellowstone Area the potential exists for contact between these species. Further disease surveys need to be conducted before any management decisions on transmission potential are determined.

Pasteurella haemolytica biotype T has been associated with disease in feeder lambs (Gilmour, 1980) and bighorn sheep (*Ovis canadensis*) (Onderka and Wishart, 1984). Most biotype T isolates recovered from bison samples in this study gave strong and clear reactions in serotype 4 antiserum. This is in contrast to agglutination reactions in two or more antisera for the majority of isolates recovered from bighorn sheep (Ward et al., 1990). Biotype A serotypes most common in domestic livestock are 1 and 2 (Jaworski et al., 1993). Although neither of these serotypes were isolated from the bison, the untypable status of the isolates would necessitate use of additional biochemical characterization and genetic evaluations to further compare these isolates with untypable strains from domestic livestock.

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