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BACTERIA ISOLATED FROM NASAL AND TONSILLAR SAMPLES OF CLINICALLY HEALTHY ROCKY MOUNTAIN BIGHORN AND DOMESTIC SHEEP

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ABSTRACT: Nasal and tonsillar samples were collected from 14 free-ranging clinically healthy Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) and 10 domestic sheep (*Ovis aries*). We identified 194 bacterial isolates, including 101 from bighorn and 93 from domestic sheep. Of these isolates, 115 were gram-positive and 79 were gram-negative. *Staphylococcus* species were the most numerous gram-positive organisms and had a higher incidence in samples from domestic than from bighorn sheep. In contrast *Streptococcus* species were present in higher numbers in samples from bighorn sheep. *Pasteurella haemolytica*, the most common gram-negative bacterium, was isolated from five of five tonsillar but from none of ten nasal samples of domestic sheep, and from seven of eight tonsillar and three of ten nasal samples of bighorn sheep. Most bacteria isolated were considered opportunistic pathogens. However, of the bacteria isolated, *P. haemolytica*, *P. multocida*, and *Actinomyces pyogenes* are most frequently associated with respiratory disease.
Key words: Bighorn sheep, *Ovis canadensis*, respiratory tract, bacterial flora.

INTRODUCTION

The number of bighorn sheep (*Ovis canadensis*) in North America in the mid-1800's was estimated to be between 1.5 and 2.0 million (Buechner, 1960). Their numbers were drastically reduced between 1870 and 1880, possibly due to an outbreak of scabies (Smith, 1954). Market and subsistence hunting, loss of suitable habitat, and diseases further decreased numbers to approximately 17,500 by 1952. Hunting regulations and relocation of animals into additional favorable habitats have resulted in increasing numbers in some areas. However, epizootic disease also has caused loss of numerous animals in some herds (Marsh, 1938; Potts, 1938; Post, 1962; Onderka and Wishart, 1984).

Deaths associated with epizootic disease of bighorn sheep has been attributed to lungworm (*Protostrongylus* sp.) infestations (Buechner, 1960) psoroptic mange (Smith, 1954), and respiratory infections associated with parainfluenza-3 virus (Parks et al., 1972), respiratory syncytial virus (Spraker et al., 1986), and various bacteria. *Actinomyces pyogenes* (Marsh, 1938), *Mycoplasma arginini* (Al-Aubaidi et al., 1972), and *Pasteurella* spp. (Marsh, 1938; Potts, 1938; Post, 1962; Onderka and Wishart, 1984) have been detected in lungs of bighorn sheep with pneumonia. Although *P. haemolytica* is a common commensal in the tonsillar region of clinically normal bighorn sheep (Dunbar et al., 1990; Ward et al., 1990), this organism also has been incriminated as a major cause of deaths in bighorn sheep (Onderka and Wishart, 1988; Foreyt, 1989). It has been speculated that transmission of these organisms from domestic to bighorn sheep results in disease and subsequent reduced survival of lambs in free-roaming bighorn sheep populations (Foreyt, 1990). Minimal information is available regarding the incidence of these and other bacteria on the mucosa of the upper repiratory tract of normal animals.

Our objective was to characterize and compare the composition of the bacterial flora in the upper respiratory tract of clinically healthy domestic and bighorn sheep.

TABLE 1. Gram-positive bacteria isolated from nasal and tonsil samples of bighorn (BH) and domestic sheep (DS).

Bacteria	Number of samples and animals culture positive[a]					
	BH nasal (n = 10)	BH tonsil (n = 8)	BH total (n = 14)	DS nasal (n = 10)	DS tonsil (n = 5)	DS total (n = 10)
Actinomyces pyogenes	0	1	1	0	0	0
Actinomycetes	2	0	2	0	0	0
Bacillus spp.	5	3	8	4	3	4
Corynebacterium spp.	3	0	3	0	0	0
Staphylococcus						
aureus	0	0	0	1	0	1
auricularis	0	2	2	0	0	0
cohnii	1	0	1	0	0	0
epidermidis	1	0	1	0	0	0
sciuri	1	0	1	2	1	3
simulans	0	0	0	1	1	1
warneri	1	0	1	1	0	1
xylosus I	0	0	0	3	0	3
xylosus II	2	1	3	5	2	5
Non-speciated	0	0	0	3	2	4
Streptococcus						
acidominimus	1	2	2	0	0	0
bovis	1	2	2	0	0	0
mitis	1	0	1	0	0	0
mutans	1	1	2	0	0	0
suis I	2	3	3	0	0	0
sanguis	1	2	3	0	0	0
Group E	0	1	1	0	0	0
Non-speciated	0	0	0	1	3	3

[a] Nasal samples only were cultured from six bighorn and five domestic sheep. Both nasal and tonsil samples were cultured from four bighorn and five domestic sheep. Tonsil samples only were cultured from four bighorn sheep.

This knowledge is essential to provide background information on the incidence and distribution of commensal and potentially pathogenic organisms in these two animal species.

MATERIALS AND METHODS

Fourteen free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were captured; ten were taken in central Idaho (USA) between 45°05′ and 45°20′N latitude and 114°20′ and 114°58′W longitude, and four were from Wyoming (USA) at approximately 43° latitude and 109°50′ longitude. The animals were captured by use of a net gun (Coda Enterprises, Inc., Mesa, Arizona, USA) or a projectile dart fired from a helicopter (Dunbar et al., 1990). Animals located in central Idaho were captured as part of a herd health survey. The four Wyoming animals were captured for relocation to Idaho's Hell's Canyon and were tested to meet Idaho state health regulations.

Nasal swabs and tonsil biopsies were obtained from the Idaho bighorn sheep as described by Dunbar et al. (1990). Specimens were collected from 10 clinically healthy domestic sheep at the University of Idaho, Caine Veterinary Teaching and Research Center (CVTRC), Caldwell, Idaho (n = 5), Fairbanks, Alaska (USA) (n = 5). Swab samples were obtained from the nasal passages of all sheep with swabs (Culturette, Marion Laboratories, Inc., Kansas City, Missouri, USA). Tissue containing the complete tonsillar crypts were collected after slaughter from the Alaska sheep. The tissues arrived within 24 hr from the time of slaughter and samples were taken by rotating a swab in the tonsillar crypts.

All samples were cultured on Columbia Blood agar (Becton Dickenson Microbiology Systems, Cockeysville, Maryland, USA) with 5% added citrated sheep blood and a *Pasteurella* selective blood agar (Ward et al., 1986). The inoculated media were incubated at 37 C in an atmosphere containing 10% CO_2 and examined daily for 5 days for bacterial growth.

An isolated colony representative of each bacterial variant detected visually was selected and identified according to methods of Carter and

TABLE 2. Gram-negative bacteria isolated from nasal and tonsil samples of bighorn (BH) and domestic sheep (DS).

Bacteria	Number of samples and animals culture positive[a]					
	BH nasal (n = 10)	BH tonsil (n = 8)	BH total (n = 14)	DS nasal (n = 10)	DS tonsil (n = 5)	DS total (n = 10)
Acinetobacter						
lwoffi	2	0	2	0	0	0
Non-speciated	0	0	0	1	0	1
Actinobacillus actinomycetemcomitans	0	1	1	1	1	1
Aeromonas						
hydrophila	0	0	0	1	0	1
Non-speciated	0	0	0	0	1	1
Enterobacter						
agglomerans	3	1	3	5	2	6
cloaca	0	0	0	2	0	2
Non-speciated	0	0	0	0	2	2
CDC IV C-2	0	1	1	0	0	0
Neisseria						
denitrificans	0	4	4	1	4	4
elongata elongata	2	1	2	0	0	0
Non-speciated	0	0	0	2	0	2
Moraxella (*Branhamella*)						
cuniculi	1	4	5	0	0	0
ovis	1	1	1	2	1	2
Pasteurella						
haemolytica T	2	6	8	0	5	5
haemolytica 3	0	0	0	0	3	3
haemolytica A	1	1	1	0	0	0
multocida	1	1	1	0	0	0
Pseudomonas fluorescens	1	0	1	0	0	0
Pseudomonas sp.	0	1	1	0	0	0
Salmonella arizonae	0	0	0	1	0	1

[a] Nasal samples only were cultured from six bighorn and five domestic sheep. Both nasal and tonsil samples were cultured from four bighorn and five domestic sheep. Tonsil samples only were cultured from four bighorn sheep.

Cole (1990), Krieg and Holt (1984), and Sneath et al. (1986). In addition Rapid STREP, STAPH Trac and 20E strips (Analytab Products, Plainview, New York, USA) were used to identify *Streptococcus*, *Staphylococcus*, and gram-negative isolates, respectively.

RESULTS

A total of 194 bacterial isolates; 101 from bighorn and 93 from domestic sheep, were evaluated. Most (n = 115) isolates were gram-positive; 79 isolates were gram-negative (Tables 1 and 2).

Thirty-one gram-positive isolates were *Staphylococcus*, twenty-two from domestic and nine from bighorn sheep. Most *Staphylococcus* (n = 25) were composed of nine species. *Staphylococcus aureus*, *S. simulans* and *S. xylosus* I, were isolated only from domestic sheep. Three species; *S. auricularis*, *S. cohnii* and *S. epidermidis*, were isolated from nasal samples of bighorn sheep only. The three remaining species; *S. sciuri*, *S. warneri* and *S. xylosus* II, were isolated from both domestic and bighorn sheep. Five isolates from domestic sheep could not be identified to species by the STAPH Trac system.

The second largest group of gram-positive organisms was *Streptococcus*. Most (18 of 22) isolates were from bighorn sheep and were identified in six species and one

group (Group E). None of the four isolates from domestic sheep were identified to species by the Rapid STREP system or conventional biochemical test procedures.

The remaining gram-positive organisms included *Actinomyces pyogenes*, *Bacillus* spp., isolates identified as Actinomycete on the basis of morphology, and *Corynebacterium* spp.

Pasteurella haemolytica was the most common species of gram-negative bacterium. Biotype T *P. haemolytica* was isolated from tonsil samples of six and nasal samples of two bighorn sheep and all five tonsil samples from domestic sheep. In addition, biotype 3 *P. haemolytica* organisms were isolated from tonsil samples of three domestic sheep and biotype A was isolated from the nasal and tonsil samples of one bighorn sheep. *Pasteurella multocida* was isolated from both the nasal and tonsillar samples from one bighorn sheep.

Other gram-negative isolates included *Acinetobacter*, *Actinobacillus*, *Aeromonas*, *Enterobacter*, *Moraxella* (subgenus *Branhamella*) *Neisseria*, and *Pseudomonas* spp., *Salmonella arizonae*, and one isolate identified with the 20E system as CDC IV C-2.

DISCUSSION

Very little information has been published regarding microflora indigenous to wild animal species, and may result in erroneous interpretation of culture results—particularly when opportunistic pathogenic bacteria are recovered from wild animals. Bacteria isolated from the upper respiratory tracts of healthy bighorn and domestic sheep in this study were identified to establish a basis for comparisons. Information regarding the habitat and pathogenesis will be discussed for those organisms which have been associated with disease.

Actinomyces species are common commensals in the oral cavity of animals. Some species, including *bovis* and *pyogenes*, also may be found on the skin (Carter and Cole, 1990). *Actinomyces pyogenes*, which was isolated from the tonsil biopsy of one of the bighorn sheep, is a ubiquitous opportunistic pathogen which may be involved in a variety of infectious conditions including peritonitis, pleuritis and mastitis (Timoney et al., 1988a).

Corynebacterium species are common in the environment, particularly on the skin of animals and the surfaces of plants (Collins and Cummins, 1986). The biochemical reactions of the three isolates recovered in this study were most characteristic of *C. striatum*, which has not been associated with diseases of animals.

Staphylococcus species are common commensals on the skin and mucous membranes of homeotherms (Kloos and Schleifer, 1986). This group of bacteria was more common in cultures from domestic sheep than from bighorn sheep. Most species are non-pathogenic and may help to prevent colonization of the skin by other potential pathogens. *Staphylococcus aureus*, an opportunistic pathogen, is most commonly associated with humans. This species also is a major cause of mastitis in cattle. Close association of humans and cattle may have resulted in the adaptation of human strains to colonization of cattle and may also explain the origin of the two isolates of *S. aureus* from domestic sheep (Kloos and Schleifer, 1986). *Staphylococcus epidermidis*, isolated from the nasal swab of one bighorn sheep, generally is isolated from humans and animals in association with humans (Kloos and Schleifer, 1986). However, both *S. aureus* and *epidermidis* were isolated from samples collected from desert bighorn sheep (Marshall et al., 1983). In contrast to those commonly isolated from humans, *xylosus* I and II which were isolated in this study, rarely are detected on the skin of humans but detected frequently from environmental samples and the skin of animals (Kloos and Schleifer, 1986). Rare infections due to *xylosus* have been reported in both humans and animals (Kloos and Schleifer, 1986).

One of the most apparent differences in the bacterial flora from the two sheep species was the number ($n = 18$) of *Streptococcus* isolates cultured from bighorn sheep in contrast to the low number ($n = 4$) from domestic sheep. *Streptococcus bovis*, a common commensal of the alimentary tract of humans and ruminants found in dairy products (Hardie, 1986), was isolated from nasal and tonsil samples of bighorn sheep. It has been reported in sporadic cases of human endocarditis (Hardie, 1986) and bovine mastitis (McDonald and McDonald, 1976). Various serotypes of *Streptococcus suis* are recognized as opportunistic causes of respiratory disease, endocarditis and encephalitis in pigs (Higgins et al., 1990) and endocarditis in humans (Clifton-Hadley, 1983). Although *S. suis* was the most common *Streptococcus* sp. isolated from bighorn sheep, diseases due to this species of bacterium have not been reported in sheep.

Actinobacillus actinomycetemcomitans, isolated from one bighorn and one domestic sheep, is a common commensal of the upper alimentary and respiratory tracts of healthy animals (Phillips, 1981). This organism causes a variety of infections including endocarditis in humans (Page and King, 1966) and epididymitis of rams (Scanlan et al., 1989).

Moraxella species exist as commensals on the mucosa of the respiratory tract of warm blooded animals (Krieg and Holt, 1984). *Moraxella* (*Branhamella*) *cuniculi* was isolated from four samples of bighorn but no domestic sheep. However, *M.* (*Branhamella*) *ovis* was isolated from equal numbers of animals in both groups of sheep. *Moraxella* (*Branhamella*) *ovis* has been incriminated as a cause of conjunctivitis and keratitis (Bovre and Hagen, 1981).

Salmonella species have a broad host range and frequently cause enteritis in animals (LeMinor, 1984). *Salmonella arizonae*, isolated from the nasal sample of one domestic sheep, commonly is detected in the feces of domestic sheep (Edwards et al., 1959). Some serotypes have been incriminated as causes of enteritis and abortion of sheep (Greenfield et al., 1973), while others appear to be non-pathogenic (Gates et al., 1979).

Pasteurella species are common commensals of the upper respiratory tracts of a variety of animals in which they also may act as opportunistic pathogens (Alley, 1975). The diseases caused by *P. multocida* and *P. haemolytica* have been characterized in domestic sheep and cattle (Timoney et al., 1988b). It appears that these organisms also act as opportunistic pathogens in bighorn sheep. *Pasteurella* species can be isolated from most clinically healthy bighorn sheep when samples are appropriately collected and preserved prior to culture (Wild and Miller, 1991).

Although this study involved small numbers of samples from the two sheep species, it provided a nucleus of information regarding bacteria encountered in the upper respiratory tract of healthy representatives of these animals. The results of cultures from Idaho bighorn sheep are very similar to those of Marshall et al. (1983), particularly in regard to the relative numbers of gram-positive bacteria. None of the bacteria isolated from either ovine species in our study, with the possible exception of *S. arizonae*, commonly act as primary pathogens; however, *Actinomyces* and *Pasteurella* species are recognized opportunistic pathogens capable of causing disease if they pass the normal defenses of the host (A.C.S. Ward, unpubl.).

Some bacteria may express either synergistic or inhibitory activity for other bacteria (Crowe et al., 1973; Corbeil et al., 1985). Corbeil et al. (1985) found that in vitro growth of members of the Pasteurellaceae was inhibited or enhanced by a variety of other bacteria isolated from the upper respiratory tract of cattle. They found that most of the Pasteurellaceae were inhibited by *Bacillus* spp. but many were enhanced by other bacteria including iso-

lates of *Acinetobacter*, *Corynebacterium*, *Micrococcus*, *Moraxella*, and *Staphylococcus*. Bacteria such as *Streptococcus* and *Staphylococcus* species, which vary in incidence in the two sheep species in our study, may influence the ability of other organisms, such as *Pasteurella*, to colonize and cause disease. Although in vitro studies cannot be assumed to provide evidence for identical in vivo association between bacteria, they do give cause for further evaluation. Additional information is needed to provide greater insight into factors which contribute to the ability of *Pasteurella* and other organisms to produce disease in bighorn sheep.

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