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Bacteria and Nematodes in the Conjunctiva of Mule Deer from Wyoming and Utah

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ABSTRACT: Swabs of conjunctiva were collected from 44 live and 226 hunter-harvested mule deer (*Odocoileus hemionus*) from Wyoming and Utah (USA). We identified 29 Gram negative and 22 Gram positive bacterial taxonomic categories, but many isolates from hunter-harvested animals were environmental contaminants. *Staphylococcus* spp. and *Micrococcus* spp. were the most common Gram positive bacteria isolated, and *Enterobacter* spp., *Escherichia coli,* and *Pseudomonas* spp. were common Gram negative bacteria isolated. *Thelazia californiensis* were found in 15% of hunter-harvested deer in Utah in 1994 and in 8% in 1995. Nematodes were found in 40% of live deer in 1995 and 66% in 1996. Three live animals showed clinical signs of infectious keratoconjunctivitis (IKC) in 1996, but pathogenic bacteria were not isolated from these individuals. Hemolytic, non-piliated *Moraxella ovis* was isolated from two clinically normal live deer in 1996 and isolates were similar to those cultured from IKC cases from Wyoming and Utah.

Key words: Bacterial flora, conjunctiva, infectious keratoconjunctivitis, mule deer, *Odocoileus hemionus.*

Infectious keratoconjunctivitis (IKC) has been extensively studied in cattle (Brown et al., 1998) and domestic sheep (Dagnall, 1994a, b), but little is known in free-ranging ruminants (e.g., Loison et al., 1996; Taylor et al., 1996). Infectious keratoconjunctivitis can cause temporary blindness in affected individuals, increasing the likelihood of predation and fatal accidents due to impaired vision. *Moraxella bovis* causes IKC in cattle (Pugh and Hughes, 1972), while *M. ovis, Chlamydia psittaci,* and *Mycoplasma conjunctivae* cause IKC in domestic sheep (Dagnall, 1994a). The etiologies of IKC in wild animals vary among species affected and differ with geographic location, but clinical signs often develop in fall and winter (Meagher et al., 1992; Taylor et al., 1996). *Moraxella* spp. have been implicated in cases of IKC in moose (*Alces alces*), pronghorn antelope (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*) from the western United States (Thorne, 1982). *Moraxella ovis* was isolated from IKC cases in moose and mule deer in Idaho and Wyoming (Dubay et al., 2000), and *M. bovis* was isolated from one clinically affected moose from Saskatchewan (Kuiken et al., 1997). Taylor et al. (1996) isolated *Moraxella* sp. from a clinically affected mule deer from Zion National Park (Utah) during the winter. *Chlamydia psittaci* also was isolated from two individuals with IKC and *Thelazia californiensis,* a parasitic nematode, was present in the conjunctival sacs of six of seven affected deer. In addition, *C. psittaci* and *M. conjunctivae* cause IKC in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) and chamois (*Rupicapra rupicapra*), respectively (Meagher et al., 1992; Loison et al., 1996). Our objective was to identify the bacterial species that occur naturally in the conjunctiva of mule deer and to compare the isolates to etiologic agents of IKC.

Hunter-harvested mule deer were sampled from check stations near Laramie (Wyoming, USA; 41°19′N, 105°35′W) and near St. George (Utah, USA; 37°06′N, 113°34′W) during October 1994 and 1995. A total of 226 adult male mule deer were sampled, 98 from Utah and 128 from Wyoming. Swabs (Culturettes, Baxter Scientific, MacGaw, Illinois, USA) of conjunctiva under the nictitating membrane or portions of nictitating membrane were taken to test for bacterial species, with particular interest in *Moraxella* spp., *Chlamydia* sp., and *Mycoplasma* spp. Isolation

methods are described in detail elsewhere (Dubay et al., 2000). All isolates from the 1994 collections were identified to species (n = 138), whereas only potential pathogens were identified in 1995. As a result, potential pathogens were isolated from 226 deer. Samples of *Chlamydia psittaci* and *Mycoplasma pulmonis* (# 19612, American Type Culture Collection, Rockville, Maryland, USA) served as positive controls for technique and were run concurrently with the samples. *Moraxella ovis* isolates were examined for piliation (Dubay et al., 2000).

Nematode parasites were collected from the conjunctiva and placed in vials containing 70% ethanol and identified based on morphological characteristics (Kofoid et al., 1937). Voucher specimens were previously deposited in the U.S. National Parasite Collection (Beltsville, Maryland, USA; Taylor et al., 1996; *Thelazia* sp., Accession number USNPC 85460).

Mule deer were captured and sampled from Zion Canyon in Zion National Park (Utah, USA; 37°18′N, 113°03′W). Twelve males (three fawns) and eight females (one fawn) were captured in January 1995 and three males and 21 females (three fawns), were sampled in January 1996. Animals were captured by drop net, swabs of the conjunctiva were taken, eyes were examined for signs of IKC, and *Thelazia* sp. were collected. Swabs for *Chlamydia* sp. isolation attempts and for isolation of other bacterial species were taken; the swab for bacterial culture was handled as for hunter-harvested deer, and the swab for *Chlamydia* sp. isolation was placed in Bovarnick's media containing gentamycin (Bovarnick, 1950). In addition, deer sampled in 1996 were tested for *Mycoplasma* sp. All bacteria isolated from deer captured in 1995 were identified, but only potentially pathogenic species were identified in 1996. As a result, pathogenic isolates were identified from 44 live deer while all bacteria were identified from 20 live deer.

Bacteria were not isolated from thirty-eight (28%) of 138 hunter-harvested deer sampled in October 1994 and four (20%) of 20 live deer sampled in January 1995. Forty-nine bacterial taxonomic categories (29 Gram negative and 22 Gram positive) were identified from conjunctival swabs from hunter-harvested or live deer (Tables 1 and 2). Certain isolates found only in hunter-harvested deer were grouped by genus. *Enterobacter* spp., *Escherichia coli* and *Pseudomonas* spp. were the most common Gram negative bacteria isolated. *Staphylococcus* spp. were the most common Gram positive isolates. *Micrococcus* spp., *Streptomyces* spp. and *Bacillus* spp. also were isolated from numerous deer. *Thelazia californiensis* were found in nine (15%) of 60 hunter-harvested deer sampled in Utah in 1994 and from three (8%) of 38 animals sampled in 1995. *Thelazia californiensis* were not seen in animals sampled from check stations in Wyoming. Non-hemolytic, non-piliated *M. bovis* was isolated from one live and one hunter-harvested animal, while non-piliated, hemolytic *M. ovis* was cultured from two live animals. *Chlamydia* sp. and *Mycoplasma* sp. were not isolated.

Clinical signs of IKC were not observed in any deer captured in 1995. In 1996, one adult female had conjunctival hyperemia, corneal opacity, blepharospasm, and epiphora, while two adult males showed corneal opacity and blepharospasm. Pathogenic bacteria were not isolated from these individuals, but non-piliated *M. ovis* was isolated from a clinically normal adult female and a female fawn (5% of deer sampled), indicating that *M. ovis* was present in the deer population at time of sampling. A non-hemolytic, non-piliated *M. bovis* was also cultured from one live and one hunter-killed animal. *Thelazia californiensis* were found in eight (40%) of 20 deer captured in 1995 and in 16 (66%) of 24 animals in 1996.

Spradbrow (1968) conducted a survey of bacterial flora of the conjunctival sac of healthy domestic sheep. *Moraxella ovis* was the most common bacterium isolated from the conjunctival sacs of sheep, but

TABLE 1. Gram negative bacteria isolated from the conjunctiva of live and hunter-harvested mule deer from Wyoming and Utah, 1994.

Bacterial species	Number (%) of isolates from live deer (n = 20)	Number (%) of isolates from hunter-harvested deer (n = 138)
Acinetobacter spp.	0 (0%)	5 (4%)
Aeromonas spp.	0 (0%)	2 (1%)
Aeromonas hydrophila	2 (10%)	0 (0%)
Actinobacillus sp.	0 (0%)	1 (1%)
Agrobacterium radiobacter	1 (5%)	0 (0%)
Chromobacterium violaceum	2 (10%)	0 (0%)
Citrobacter freundii	0 (0%)	2 (1%)
Eikenella corrodens	2 (10%)	0 (0%)
Enterobacter agglomerans	5 (25%)	10 (7%)
Enterobacter cloacae	2 (10%)	2 (1%)
Enterobacter faecalis	1 (5%)	3 (2%)
Escherichia coli	0 (0%)	10 (7%)
Escherichia vulnaris	1 (5%)	0 (0%)
Flavobacterium spp.	1 (5%)	5 (4%)
Kingella spp.	0 (0%)	3 (2%)
Klebsiella pneumoniae	0 (0%)	1 (1%)
Moraxella bovis (non-hemolytic)	1 (2.3%)[a]	1 (0.4%)[b]
Moraxella ovis	2 (4.5%)	0 (0%)
Pasteurella spp.	0 (0%)	5 (4%)
Proteus spp.	0 (0%)	8 (6%)
Pseudomonas spp.	0 (0%)	8 (6%)
Pseudomonas maltophila	2 (10%)	6 (4%)
Pseudomonas paucimobilis	1 (5%)	1 (1%)
Pseudomonas stutz	1 (5%)	1 (1%)
Psychrobacter immobilis	0 (0%)	1 (1%)
Serratia marsescens	1 (5%)	0 (0%)
Vibrio mimicus	0 (0%)	2 (1%)
Weeksella zoohelcum	0 (0%)	2 (1%)
Yersinia enterocolitica	0 (0%)	1 (1%)

[a] Sample size of 44 live deer.
[b] Sample size of 226 hunter-harvested deer.

Micrococcus spp., *Streptococcus* spp., *Bacillus* spp., *Moraxella* spp. and *Corynebacterium* spp. were also isolated. Dagnall (1994b) also sampled the conjunctival sacs of clinically healthy sheep, and isolated bacteria from 11 of 37 eyes sampled. *Staphylococcus* spp. were the most common bacteria isolated, but *M. ovis, Streptomyces* spp., *Streptococcus* spp., and *Bacillus* spp. were also present. Both authors suggested that *M. ovis* naturally occurs in the conjunctival sacs of sheep, but it may contribute to IKC when predisposing factors are present, such as ultraviolet light or trauma. Similar bacteria to those isolated from sheep were recovered from hunter-harvested and live mule deer from Wyoming and Utah (Tables 1 and 2).

Moraxella spp. are commensals of mucous membranes of animals and may cause IKC. *Moraxella* sp., *M. bovis,* and *M. ovis* were isolated previously from IKC cases in free-ranging ruminants (Taylor et al., 1996; Kuiken et al., 1997; Dubay et al., 2000). In contrast, Webber and Selby (1981) did not isolate *Moraxella* spp. from 293 healthy hunter-harvested white-tailed deer (*O. virginianus*) and researchers did not attempt to identify other bacteria. The *M. bovis* isolates from mule deer in Wyoming and Utah were non-hemolytic, suggesting that they may be non-pathogenic com-

TABLE 2. Gram positive bacteria isolated from the conjunctiva of live and hunter-harvested mule deer from Wyoming and Utah, 1994.

Bacterial species	Number (%) of isolates from live deer (n = 20)	Number (%) of isolates from hunter-harvested deer (n = 138)
Aerococcus spp.	0 (0%)	4 (3%)
Actinomyces sp.	0 (0%)	1 (1%)
Arthrobacter histidinolovorans	0 (0%)	4 (3%)
Arthrobacter simplex	0 (0%)	6 (4%)
Bacillus spp.	2(10%)	29 (21%)
Cellulomonas spp.	0 (0%)	5 (4%)
Corynebacterium spp.	0 (0%)	14 (10%)
Erysipelothrix rhusiopathiae	0 (0%)	1 (1%)
Gemella haemolysans	0 (0%)	1 (1%)
Kurthia spp.	0 (0%)	2 (1%)
Lactobacillus lactis	0 (0%)	1 (1%)
Microbacterium arborescens	0 (0%)	1 (1%)
Micrococcus spp.	8 (40%)	24 (17%)
Rhodococcus spp.	0 (0%)	8 (6%)
Staphylococcus spp.	0 (0%)	30 (22%)
Staphylococcus auricularis	4 (20%)	7 (5%)
Staphylococcus capitis	4 (20%)	4 (3%)
Staphylococcus cohnii	2 (10%)	5 (4%)
Staphylococcus xylosus	1 (5%)	4 (3%)
Streptococcus faecalis	3 (15%)	0 (0%)
Streptococcus spp.	0 (0%)	4 (3%)
Streptomyces spp.	2 (10%)	12 (9%)

mensals since hemolysin production is correlated with virulence (Beard and Moore, 1994).

However, isolates of hemolytic *M. ovis* from live deer closely resembled those from cases of IKC in moose and mule deer from Wyoming (Dubay et al., 2000). Therefore, it is possible that *M. ovis* contributed to IKC in deer from Zion National Park because the bacterium was present in the population at time of sampling. Deer with conjunctival and corneal lesions were observed during the fall and winter of 1996. However, *M. ovis* was not isolated from three individuals showing clinical signs. It is possible that another organism, such as *Mycoplasma* sp. or *C. psittaci,* was responsible for IKC in these deer and was not isolated due to difficulty in isolating these organisms. *Moraxella ovis* was isolated from one clinically normal fawn and one normal adult female; perhaps the deer with *M. ovis* were sampled before or after clinical disease, were immuno-competent enough to deter clinical disease, or were asymptomatic carriers of the bacterium.

Several of the bacterial isolates were only cultured from hunter-harvested animals, suggesting that they colonized the eyes post-mortem or were contaminants due to handling after the animals were killed. Many of the Gram negative bacteria isolated from the conjunctiva were environmental contaminants (Kreig and Holt, 1984). For instance, *Enterobacter* spp. are commonly isolated from animal and human feces, *Pseudomonas* spp. are cultured from plants and soil, and *Aeromonas* spp. are found in water and sewage. The Gram positive bacteria isolated from conjunctival swabs are considered contaminants or normal flora of mammalian skin (Sneath et al., 1994).

The eye nematode, *T. californiensis,* has been found in several wild species including black-tailed deer (*O. h. columbianus*; Beitel et al., 1974). *Thelazia* spp. are often found in clinically normal animals (Souls-

by, 1982), and Beitel et al. (1974) reported the presence of *T. californiensis* in 33% of deer sampled in northwest Oregon. Gross lesions were not seen in infected animals.

In conclusion, *M. ovis* does not appear to be a primary pathogen in mule deer, but it may have contributed to IKC in Zion National Park. In addition, many bacteria isolated from conjunctival swabs of live and hunter-harvested mule deer were environmental contaminants or normal flora of the conjunctival sac.

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