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## ASSOCIATION OF *MORAXELLA OVIS* WITH KERATOCONJUNCTIVITIS IN MULE DEER AND MOOSE IN WYOMING

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**ABSTRACT:** Six cases of infectious keratoconjunctivitis (IKC) in mule deer (*Odocoileus hemionus*) and moose (*Alces alces*) in Wyoming (USA) were investigated during fall and winter of 1995 and 1996. Excessive lacrimation, mucopurulent conjunctivitis, keratitis, and corneal opacity were observed in mule deer. Moose had severe mucopurulent conjunctivitis, keratitis, and corneal ulceration. Hemolytic, non-piliated *Moraxella ovis* was isolated from two mule deer and two moose. We attempted to reproduce IKC in three mule deer fawns using an isolate of *M. ovis* from a clinically affected mule deer. These fawns did not develop clinical signs of infection and the bacterium was not reisolated from inoculated deer. Inoculated deer may not have developed clinical signs because deer were not exposed to ultraviolet light or mechanical insult before inoculation. In addition, the isolate used for inoculation may have lost virulence factors through passage, or *M. ovis* may not have been the primary pathogen responsible for clinical disease in the natural cases of IKC we investigated. The etiology of IKC in free-ranging wild ruminants remains poorly understood.

**Key words:** *Alces alces*, keratoconjunctivitis, moose, *Moraxella ovis*, Mule deer, ocular disease, *Odocoileus hemionus*, pinkeye.

### INTRODUCTION

Infectious keratoconjunctivitis (IKC) causes blepharospasm, epiphora, corneal opacity and ulceration, and conjunctivitis in domestic and wild ruminants (Thorne, 1982; Hosie, 1988; Iwasa et al., 1994; Moore, 1996). *Moraxella bovis* is the major cause of infectious bovine keratoconjunctivitis, a disease of economic importance worldwide (Baldwin, 1945; Moore, 1996). *Moraxella ovis* has been implicated in epizootics of IKC in domestic sheep and goats (Bulgin and Dubose, 1982; Dagnall, 1994a), and cattle (Pedersen, 1972). Dagnall (1994b) reported that *M. ovis* could be isolated from both healthy sheep and those with IKC, but isolation occurred at a higher rate in diseased animals.

Infectious keratoconjunctivitis is a sporadic but relatively common disease of free-ranging cervids in western North America. Cases have been described in mule deer (*Odocoileus hemionus*), pronghorn antelope (*Antilocapra americana*), and moose (*Alces alces*) from Wyoming (USA), but an etiologic agent was not iso-

lated (Thorne, 1982). During fall and winter of 1989–90, an epizootic of IKC in mule deer was investigated in Idaho (USA); *M. ovis* was isolated from seven of approximately 150 hunter-harvested deer with corneal and conjunctival lesions, constituting 40 to 50% of the animals examined (D. Hunter, pers. comm.). *Moraxella ovis* was also isolated from two moose with IKC in Idaho (D. Hunter, pers. comm.). Taylor et al. (1996) isolated *Chlamydia psittaci* and *Moraxella* sp. from mule deer with IKC in Zion National Park (Utah, USA) during the winters of 1992–93 and 1993–94. Kuiken et al. (1997) investigated the cause of impaired vision in seven moose from Saskatchewan (Canada). Clinical signs included keratitis, corneal scars, and cataracts, and *M. bovis* was isolated from one animal.

In addition to *Moraxella* spp., *C. psittaci* and *Mycoplasma conjunctivae* cause IKC in sheep and goats (Wilsmore et al., 1990; Van Halderen et al., 1994). Meagher et al. (1992) implicated *C. psittaci* in an IKC epizootic in Rocky Mountain bighorn

TABLE 1. Cases of infectious keratoconjunctivitis in free-ranging cervids from Wyoming, 1995–96.

Species	Date	County (latitude and longitude)	Sex	Age	Isolates
Moose	10/12/95	Lincoln (42°40'N, 110°58'W)	Male	2 yr	Contaminants
Moose	10/16/95	Sweetwater (41°39'N, 108°05'W)	Male	2 yr	<i>Moraxella ovis</i>
Moose	11/6/95	Teton (43°40'N, 110°45'W)	Female	Adult	<i>Moraxella ovis</i>
Mule deer	12/1/95	Park (44°08'N, 108°52'W)	Male	Adult	<i>Moraxella ovis</i>
Mule deer	1/4/96	Sublette (42°52'N, 109°52'W)	Male	Adult	<i>Moraxella ovis</i>
Mule deer	1/31/96	Sublette (42°52'N, 109°52'W)	Male	1 yr	Contaminants

sheep (*Ovis canadensis canadensis*) which contributed to 60% mortality in the herd. An epizootic of IKC in chamois (*Rupicapra rupicapra*) in the northern French Alps was attributed to *M. conjunctivae* (Loison et al., 1996), and *M. conjunctivae* was isolated from an epizootic of IKC in ibex (*Capra ibex*) in Switzerland as well (Mayer et al., 1997).

Experimentally inducing IKC with *M. bovis* in cattle is difficult and exposing eyes to ultraviolet light before inoculation is often necessary (Hughes et al., 1965, 1977). In order to induce IKC in mice, corneas must be abraded with a wire brush before inoculation as well (Gerber and Frank, 1983).

Our objectives in the present study were to determine the etiology of IKC cases in free-ranging mule deer and moose presented for diagnosis at the Wyoming State Veterinary Laboratory (WSVL), Department of Veterinary Sciences, University of Wyoming (Laramie, Wyoming), and to determine if isolates of *M. ovis* were pathogenic in mule deer.

#### MATERIALS AND METHODS

During the fall and winter of 1995–96, six cases of IKC in free-ranging ruminants from different counties in Wyoming were investigated (Table 1). Conjunctivae were swabbed beneath the nictitating membrane using cotton-tipped swabs (Culturette, Baxter Scientific, McGaw Park, Illinois, USA). These were used

for mycoplasmal and routine bacterial culture. Those for *Mycoplasma* isolation were placed in 3 ml vials of *Mycoplasma* medium (Bacto-Mycoplasma Supplement S broth, Difco Laboratories, Detroit, Michigan, USA). Vials were incubated at 37 C in a 5% CO<sub>2</sub>-enriched atmosphere for approximately 48 hr and seven serial 10-fold dilutions were made to dilute past contaminant bacteria. Vials were incubated for an additional 7 to 10 days and the first dilution which showed little or no turbidity, indicating lack of bacterial contamination, was plated onto *Mycoplasma* medium (Bacto-Mycoplasma Supplement S agar, Difco Laboratories) and further incubated for 7 to 10 days. Plates were then examined for “fried-egg” shaped colonies with a dissecting scope. A stock culture of *Mycoplasma pulmonis* (American Type Culture Collection # 19612, Rockville, Maryland, USA) was grown in a parallel manner as a control for technique.

Conjunctival swabs for bacterial culture were plated on Columbia blood agar (CBA) and MacConkey (MAC) agar plates (MicroBio Products, Inc., Phoenix, Arizona, USA). Since virulent strains of *M. bovis* are often  $\beta$ -hemolytic (Pugh and Hughes, 1968), Gram negative bacteria showing  $\beta$ -hemolysis were recultured and identified to species using the BIOLOG identification system (BIOLOG, Hayward, California, USA) and associated tests (Fraser and Gilmour, 1979). BIOLOG was used to identify bacteria to genus. When an isolate was identified as *Moraxella* spp., the isolate was examined by darkfield microscopy and a nitrate reduction test (Difco Laboratories) was conducted to differentiate between *M. bovis* and *M. ovis* (Fraser and Gilmour, 1979).

All *M. ovis* isolates were examined for pili via transmission electron microscopy. Bacterial iso-

lates were grown on CBA and the plates were flooded with phosphate buffered saline (pH 9.2). The bacteria were then placed on a C-C-copper 300 mesh grid (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA). The grids were washed five times in water droplets. They were then coated with platinum/palladium (80:20 at 15° angle), and examined under a transmission electron microscope at 63,000× (Bradley, 1967). Electron micrographs were compared to those of piliated *M. bovis* and *M. catarrhalis* isolates (Marrs and Weir, 1990). A known piliated isolate of K99 *Escherichia coli* was also prepared and examined in the same manner as a control for piliation.

*Chlamydia psittaci* isolation attempts were conducted by the method of Schacter and Dawson (1979) and Brown and White (1982), as modified by Taylor et al. (1996). Portions of cornea and conjunctiva were ground in a tissue grinder with Bovarnick's medium (Bovarnick et al., 1950), and the fluid was inoculated onto plates of McCoy cells (rabbit kidney, American Type Culture Collection). Detection of *Chlamydia* sp. was conducted by determining cytopathic effect on the cells and by fluorescent antibody (National Veterinary Services Laboratory, Ames, Iowa, USA) examination. Control cultures of *C. psittaci* and wells containing only media served as positive and negative controls and were run concurrently with the samples.

After samples were collected for microbiology, the eyes were trimmed of surrounding soft tissues and extraocular muscles and fixed in 10% phosphate buffered formalin. Sagittal sections of the globes were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy.

Four healthy hand-reared 9-mo-old mule deer fawns, two males and two females, were obtained from the Sybille Wildlife Research and Conservation Education Center (Sybille), Wyoming Game and Fish Department (Wheatland, Wyoming, USA). Infectious keratoconjunctivitis was not observed in the fawns or other captive cervids while they were housed at Sybille. Three fawns were housed separately from a single fawn in two rooms of an isolation facility (WSVL) for 2 wk prior to inoculation and for 4 wk afterward. Blood was drawn via jugular venipuncture and sera were tested for presence of antibodies against *C. psittaci* via complement fixation using the modified procedure of Karrer et al. (1950; Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, USA). An antibody titer > 1:16 was considered positive of exposure to *Chlamydia* sp.

*Moraxella ovis* isolated from the conjunctiva

of an adult male mule deer with severe IKC was used as the inoculum. The isolate was obtained in December 1995 and passed on CBA once or twice before being frozen in citrated sheep blood at -70 C. The isolate was grown on CBA for 24 hr and then removed from the plates and placed in sterile saline (0.85%) with a sterile swab. The inoculum contained  $6.0 \times 10^8$  colony forming units (CFU) per ml as determined by back titration and plating on CBA.

Conjunctival swabs were taken three times from all deer for bacterial and mycoplasmal cultures during the pre-inoculation period. Animals were manually restrained during inoculation. Three deer received 0.5 ml of the *M. ovis* inoculum in the left conjunctival sac with a tuberculin syringe without a needle. The eyelid was lightly held shut for approximately 30 seconds (Aikman et al., 1985). The control deer received only sterile saline. The conjunctiva and cornea of all deer were examined each day for 30 days post-inoculation. Conjunctival swabs for culture were taken from both eyes on days 1, 2, 3, 5, and 7 post-inoculation and then twice weekly for 3 wk.

## RESULTS

Gross ocular lesions observed in specimens from three moose were severe bilateral conjunctivitis and keratitis with corneal ulceration. Mule deer had excessive bilateral purulent lacrimation, mucopurulent conjunctivitis, keratitis, and corneal opacity.

Histologically, all eyes were moderately to severely autolytic and some were distorted by freezing artifact. Retinas in all eyes were artifactually detached. The mule deer eyes were similar and characterized by infiltrates of neutrophils and lymphocytes in the stroma of the cornea, particularly at the limbus or in areas of corneal ulceration, and irregular corneal epithelial hyperplasia. The corneal stroma was edematous and vascularization occurred at the periphery. A similar mixed inflammatory cell infiltrate was present in the conjunctiva. No inflammation was observed in the internal structures of affected eyes.

Microscopic lesions in moose were more severe than those observed from mule deer. The inflammatory reaction was similar but ulceration with corneal rupture and anterior synechia were present in two

moose; the lens had been lost from the eye of one of these animals. In these cases there was abundant granulation tissue and vascularization of the cornea. Anterior uveitis and hypopyon were present.

*Moraxella ovis*, in addition to several bacteria considered to be contaminants (Dubay, 1996), were cultured from the eyes of two mule deer and two moose, and only contaminants were isolated from one mule deer and one moose (Table 1). Contaminants isolated from the eyes included *Micrococcus* spp., *Bacillus* spp., *Staphylococcus* spp., *Enterobacter* spp., and *Flavobacterium* spp. Pili were not seen by electron microscopy on any *M. ovis* isolate, but the K99 *E. coli* isolate was obviously piliated. All *M. ovis* isolates were similar and were identified as such by morphologic and biochemical tests; the isolates were off-white, hemolytic, cocci, which reduced nitrate. *Moraxella bovis* was not cultured from the cases examined. Chlamydial and mycoplasmal isolation attempts were negative, but controls for cultural techniques were positive.

Clinical disease was not observed in experimentally inoculated fawns, and *M. ovis* was not isolated from conjunctival swabs prior to or during these experiments. All four fawns had *Chlamydia* sp. antibody titers of  $\leq 1:16$ , which were considered negative.

## DISCUSSION

Isolation of *M. ovis* from four clinical cases of IKC in free-ranging cervids is unusual. In the past, most isolates of *Moraxella* spp. from free-ranging cervids have been identified as *M. bovis*, *M. lacunata*, or not identified to species (Thorne, 1982; Taylor et al., 1996). This may be due to taxonomic confusion in this group of bacteria (Enright et al., 1994). It is possible that *M. ovis* isolates have been misidentified as *M. bovis* when cultured from affected cervids because morphological characteristics and nitrate reduction might not have been considered. *Moraxella ovis* has contributed to cases of IKC in sheep,

but it was not considered a primary pathogen (Wilsmore et al., 1990; Dagnall, 1994a). On the other hand, Bulgin and Dubose (1982) suggested *M. ovis* was the primary cause of IKC in an outbreak in goats. In the absence of isolation of other potential causes of IKC in these cervid cases and the apparent rarity of *M. ovis* in normal deer eyes (Dubay, 1996), it is possible that *M. ovis* influences the occurrence of IKC. Additional study is warranted.

Pathogenic bacteria were not isolated from one moose and one mule deer. Only eyes were submitted from the moose, thus bacterial contamination during excision of the eyes could have caused contaminant bacteria to grow over pathogenic bacteria, including *Moraxella* sp.. Only one eye from the deer was intact and suitable for culture, and the head had been frozen and thawed. Freezing is not optimal for recovery of some bacteria (Quinn et al., 1994), and it may have decreased the likelihood of isolating bacterial pathogens.

There are several possibilities why disease was not observed in experimentally exposed deer. First, *M. ovis* may not have been a primary pathogen in any of the cases of IKC, even though other pathogens were not isolated. Perhaps *Mycoplasma* sp. or *C. psittaci* induced disease, allowing *M. ovis* to colonize the eyes, but neither *M. conjunctivae* nor *C. psittaci* was recovered. Both *Mycoplasma* spp. and *Chlamydia* spp. are relatively difficult to culture and the condition of samples submitted from the field was less than ideal.

Virulence factors have been identified in *M. bovis*, and perhaps they were lost in passage or not present in the *M. ovis* isolate (Gillespie and Timoney, 1981; Mims, 1987). Pili are believed to allow the bacteria to adhere to corneal and conjunctival surfaces, thereby delivering toxins directly to cellular receptors (Beachey, 1981; Ruehl et al., 1993). It seems unlikely that virulence factors would have been lost after only two passages, but piliated *M. bovis* isolates can lose pili with *in vitro* passage

(Wilt et al., 1990). Our isolates were not pilated, but did produce hemolysin, another virulence factor (Clinkenbeard and Thiessen, 1991). Pugh and Hughes (1968) found that the occurrence of bovine keratoconjunctivitis was correlated with hemolytic *M. bovis*.

Experimental inoculation of *M. bovis* obtained from cases of IKC in cattle do not consistently cause IKC without exposing the eyes to ultraviolet light (Hughes et al., 1965). Also, animals exposed to ultraviolet radiation before *M. bovis* inoculation developed severe cases of IKC which resembled field cases, whereas those exposed to *M. bovis* alone developed mild lesions (Hughes et al., 1965; Pugh and Hughes, 1968). In a free-ranging situation, deer are naturally exposed to ultraviolet light, but the fawns in our study were not. Mechanical insult, such as abrading the corneas and conjunctiva to give the bacteria an entry route, was not done. In the wild, deer could naturally damage corneal and conjunctival tissue while foraging or through altercations during the rut. In addition, dust could cause mechanical damage. Our fawns were not exposed to such predisposing factors. It is possible that the fawns had been exposed to *M. ovis* prior to inoculation and developed immunity to infection, although fawns did not show signs of IKC while being hand-raised at Sybille. To our knowledge, serologic testing for antibodies against *M. ovis* is not available.

In conclusion, *M. ovis* does not appear to be the primary pathogen or sole cause of IKC in mule deer or moose, even though it was the only potential pathogen isolated from four of six cases of IKC in free-ranging cervids examined. The etiology of IKC is likely multifactorial, involving bacterial, environmental, and immunological components. Environmental and mechanical insult may be required for IKC due to *M. ovis* to develop. This sporadic and occasionally epizootic disease in free-ranging cervids may have population impacts in some wild ungulate species

(Meagher et al., 1992; Loison et al., 1996), but even limited outbreaks and isolated cases may be of concern to the public and wildlife managers (Taylor et al., 1996).

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