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INFECTIOUS KERATOCONJUNCTIVITIS IN FREE-RANGING MULE DEER (*ODOCOILEUS HEMIONUS*) FROM ZION NATIONAL PARK, UTAH

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ABSTRACT: An epizootic of infectious keratoconjunctivitis (IK) was studied opportunistically in free-ranging mule deer (*Odocoileus hemionus*) from Zion National Park, Utah (USA), from November 1992 to March 1994. *Moraxella* sp. and *Chlamydia* sp. were isolated from the conjunctiva of two of seven deer. In addition, *Thelazia californiensis* occurred on the conjunctivas of six of seven deer. Based on field observations, adults appeared to be affected clinically at a higher incidence during both years as opposed to juveniles. Corneal opacity was the most apparent clinical sign from 1992 to 1993. However, in the following year, blepharospasm and epiphora were noted more often. We were also able to document the clinical recovery of three affected deer. In addition, *Moraxella* sp. was recovered from the eyes of a clinically unaffected deer 1 year after the epizootic occurred.

Key words: Infectious keratoconjunctivitis, pinkeye, *Chlamydia* spp., *Moraxella* spp., *Thelazia californiensis*, mule deer, *Odocoileus hemionus*, Zion National Park, Utah.

INTRODUCTION

Infectious keratoconjunctivitis (IK), also known as pinkeye and vascular keratitis has been reported in domestic livestock in most countries of the world (Wilcox, 1968). In cattle, the disease is highly contagious and epizootics are most common in the warmer months, especially during humid weather (Wilcox, 1968). Predisposing factors have included dust, sunlight, and flies which mechanically transfer the organisms involved between cattle (Brown and Adkins, 1972). In cattle, prevalence is usually highest in young animals (Baptista, 1979). Various etiological agents have been implicated, including bacteria (*Moraxella* sp., *Neisseria* sp., *Listeria* sp., *Chlamydia* sp.), mycoplasmas, viruses, and nematodes (*Thelazia* sp.) (Baptista, 1979).

Few cases of IK in free-ranging wildlife are documented. Cases often are undetected because animals may be taken easily by predators. Most reports involve only individuals or small groups of animals, such as mule deer, white tailed deer (*O. virginianus*), pronghorn antelope (*Antilocapra americana*), and moose (*Alces alces*) (Thorne, 1982). Infectious keratoconjunctivitis was implicated as an indirect mortality factor in the decline of a large bighorn sheep (*Ovis canadensis*) population (Meagher et al., 1992).

Zion National Park is located in the southwestern corner of Utah (USA) (112°59′S, 37°16′W). The epizootic occurred in the Park's Zion Canyon which is approximately 5 km long and 0.5 km wide, much of the area can be seen from a road which runs the length of the canyon. The wintering Zion Canyon mule deer herd was believed to have contained 200 individuals (Vieira et al., 1994) and vegetation appeared to be of adequate quantity and quality to sustain those populations (Cunningham, 1992). We describe an epizootic of infectious keratoconjunctivitis in a free-ranging mule deer herd within Zion National Park during the winters of 1992 to 1993 and 1993 to 1994. Our objectives

were to characterize the epizootiology and identify likely etiological agents.

MATERIALS AND METHODS

The first mule deer having vision difficulty in Zion National Park was observed on 20 November 1992. Organized field observations of the deer for clinical signs began on 14 December 1992 and continued through March 1994 with a break during the summer when deer dispersed out of the canyon. The observation process entailed two resource managers traveling up and down the 5 km long Zion Canyon road and using binoculars to observe the deer for clinical signs. Over 2,600 individual deer observational reports were made. Observations for each deer included: date, sex, age (adult or juvenile), location, eye lesions (normal, epiphora, blepharospasm, corneal opacity), eye affected (unilateral or bilateral) and, when possible, individual animal identification (antler points and configuration, and torn ears). Documenting the course of the clinical signs in the free-ranging mule deer resulted in many of the deer being counted on consecutive days.

Seven deer (one adult female, three adult males, one juvenile female, and two juvenile males) in poor body condition were shot. Carcasses were examined for gross lesions. Blood, major organs (lung, liver, spleen, kidney, intestine, heart, skeletal muscle), and the head were collected and sent overnight mail to the University of Wyoming, Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA). Upon arrival, further gross examinations were conducted.

Tissues (lung, liver, spleen, kidney, intestine, heart, skeletal muscle) for histologic evaluation were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin. We tested for caprine arthritis-encephalitis/ovine progressive pneumonia antibodies with an immunodiffusion test (Veterinary Diagnostic Technology, Wheat Ridge, Colorado, USA). For bacterial isolations, conjunctival swabs were streaked on Columbia blood agar and MacConkey agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) and incubated in 5% CO_2 at 35 C for 48 hr. The identification techniques of Carter and Cole (1990) were used to identify the bacteria.

Chlamydia spp. isolations were attempted using minced cornea and conjunctiva placed in Bovarnick's medium (Bovarnick et al., 1950) containing 10 μg/ml gentamicin (Gibco Laboratories, Life Technologies, Inc., Grand Island, New York, USA). Samples were centrifuged at 340 × G, at −20 C for 15 min. The supernatant was decanted and mixed with an equal volume of medium and centrifuged as before. The resulting supernatant was stored at −70 C until attempted isolation. Samples were thawed at 37 C and inoculated onto duplicate wells of a 24 well plate (Corning, Cambridge, Massachusetts, USA) containing CRL 1696 McCoy cells (American Type Culture Collection, Rockville, Maryland) with 12 mm cover glasses. Wells containing samples were centrifuged at 2126 × G, 25 C, for 60 min, followed by incubation with minimum essential medium with Earle's salts and nonessential amino acids (Gibco Laboratories) containing 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah) at 37 C in 5% CO_2 for 7 days. McCoy cells were examined daily for cytopathic effect and stained at 7 days postinoculation with a direct fluorescent antibody stain (Cultureset Chlamydia Identification Reagent, Ortho Diagnostic Systems, Raritan, New Jersey, USA) to detect *Chlamydia* spp.

Infectious bovine rhinotrachitis (IBR) isolations were attempted using minced cornea and conjunctiva placed in Bovarnick's medium (Bovarnick et al., 1950). Samples were centrifuged at 340 × G, at −20 C for 15 min. Supernatant was decanted and mixed with an equal volume of medium and centrifuged as before. Resulting supernatant was stored at −70 C until attempted isolation. Samples were thawed at 37 C and Medium 199 with Earles salts (Gibco Laboratories) containing 2% fetal bovine serum (Sigma Chemical Co, St. Louis, Missouri, USA) to control viruses. We added BHV (bovine herpes virus) type I (National Veterinary Service Laboratory (NVSL), Ames, Iowa, USA) to confluent monolayers of fetal bovine cells (Monfort Biologicals, Greely, Colorado) in a 48 well plate (Corning). Cells inoculated with the samples then were incubated at 37 C, 5% CO_2 for 2 days at which time they were passed into tissue cultures. They then were observed daily for 7 days with an inverted light microscope. If cytopathic effects were noted, the cells were stained with IBR fluorescein isothiocyanate conjugated antiserum (FITC) (NVSL) for the presence of IBR antigen.

Direct flourscent antibody staining of conjunctival and corneal sections for IBR and *Chlamydia* sp. was conducted by placing 1 mm^2 of tissue on to a cryostat chuck with OTC embeddment compound (Miles Inc., Elkhart, Indiana, USA). The chuck then was placed in the cryostat at −10 C. Tissues were cut into 4 μm sections, placed on slides, fixed in acetone for 5 min and then allowed to air dry. We added IBR FITC conjugated antiserum (NVSL) to half of these sections, and *Chlamydia psittaci* FTIC conjugated antiserum (NVSL) to the oth-

TABLE 1. Observations of mule deer in Zion National Park affected with infectious keratoconjunctivitis, winters of 1992 to 1993 and 1993 to 1994.

	Number of observation days	Number of deer observed	Number of deer affected	Percent of all deer affected	Percent of all adults affected	Percent of all juveniles affected
1992 to 1993						
December	13	314	9	3	11	0
January	19	525	66	13	31	1
February	8	184	13	7	35	3
March	8	218	9	4	23	1
Total	48	1,241				
1993 to 1994						
November	6	289	29	10	25	5
December	11	393	49	13	32	5
January	9	531	62	12	36	3
February	5	242	24	10	24	2
Total	31	1,455				

er sections. All sections then were incubated at 37 C in 5% CO_2 for 30 min. Coverslips were applied and sections were observed microscopically for positive fluorescence.

Corneas and conjunctivas from four of the deer were randomly selected and examined by negative contrast stain electron microscopy for virus-like particles (C. Hearne, pers. comm.). Tissues were ground with a mortar and pestle, diluted with distilled water and centrifuged at 3,024 × G for 20 min. The supernatant was then centrifuged for 1 hr at 39,191 × G. The pellet was resuspended in 10 drops of distilled water. One drop of the suspension was added to a mixture of the following ingredients: 20 drops of distilled water, four drops of 4% phosphotungstic acid (Electron Microscopy Sciences, Ft. Washington, Pennsylvania, USA) and one drop of 0.1% bovine serum albumin (Gibco Laboratories). The mixture was nebulized on to collodion-coated 300 mesh copper grids. The material on the grids was then examined with a Philips 410 LS transmission electron microscope (Philips Electronic Instruments, Mahwah, New Jersey) for the presence of virus-like particles at 60 KV.

Ocular parasites were mounted in Hoyer's medium (Pritchard and Kruse, 1982) and identified based on morphological characteristics described by Kofoid et al. (1937). Representative specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland, USA) under accession number USNPC 85460.

RESULTS

Clinically, adults were observed affected at a higher prevalence than juveniles both years (Table 1). No significant difference was noted between the number of affected adult males or females. Clinical signs were observed to occur unilaterally in 47 and bilaterally in 50 deer from 1992 to 1993. The following winter (1993 to 1994), we observed 70 deer affected unilaterally and 94 bilaterally. During the first year of the epizootic, we found 194 affected deer eyes. Corneal opacity was the most frequently reported clinical sign occurring in 125 eyes with blephraspasm noted in 53 eyes and epiphora in 30 eyes. However, in the following year, as observers became more familiar with the clinical signs of the disease this trend changed and of the 164 eyes observed to be clinically affected, blepharospasm occurred in 82, epiphora in 67, and corneal opacity in 36. We were also able to document the clinical recovery of three affected deer that had identifiable characteristics. In addition, *Moraxella* sp. was recovered from the eye surface of a clinically unaffected deer 1 yr after the epizootic occurred.

Bilateral keratoconjunctivitis was noted in six of seven deer necropsied. Mild to moderate amounts of mucopurulent exudate and nematodes (*Thelazia californiensis*) were present on the conjunctiva of six of the affected deer. Corneas were cloudy with focal areas of opaqueness and slight hyperemia at the limbus. Focal ulcerations and vascularization were present.

TABLE 2. Laboratory results from seven Zion National Park mule deer affected with clinical signs of infectious keratoconjunctivitis.

Sex	Age	IBR FA[a]	Electron microscopy	*Chlamydia* isolate	Bacterial isolate	Ocular parasites
Female	Adult	Neg[b]	Neg	Pos	*Proteus* sp.	*Thelazia californiensis*
Male	Adult	Neg	ND	Neg	*Pseudomonas* sp. *Staphylococcus* sp. *Streptococcus* sp.	*Thelazia californiensis*
Male	Adult	Sus	Neg	Neg	*Proteus* sp	*Thelazia californiensis*
Male	Adult	Sus	Neg	Neg	*Proteus* sp. *Escherichia coli*	*Thelazia californiensis*
Female	Juvenile	Neg	ND	Neg	*Moraxella* sp.	*Thelazia californiensis*
Male	Juvenile	Neg	ND	Pos	No growth	Neg
Male	Juvenile	Neg	Neg	Neg	*Proteus* sp.	*Thelazia californiensis*

[a] Fluorescent antibody test for infections bovine rhinotrachites.
[b] Neg, negative; Pos, positive; Sus, suspect; ND, not done.

Microscopically, conjunctivas were essentially normal with some blood vessels lightly cuffed by lymphocytes. Corneas were characterized by thickening of the epithelium along the limbus. The corneal epithelium was intact with areas of lymphocyte aggregation, and infiltrated by plasma cells and a few neutrophils beneath the epithelium. Limbal stroma was invaded by capillaries and infiltrated by neutrophils, plasma cells, and a few melanocytes. Sections of the optic nerve, ciliary body and iris, retina, and lens were essentially normal. Lesions in the corneas and conjunctivas of two of the adult male mule deer appeared to be resolving. Only the juvenile female had no significant gross or histologic ocular lesions.

Bacterial isolates obtained from eye swabs included: *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. (coagulase negative), *Streptococcus* sp. and *Escherichia coli*. *Moraxella* sp. was recovered from both eyes of one juvenile (Table 2). Fluorescent antibody staining of frozen sections of cornea and conjunctiva from all deer, were negative for *Chlamydia* sp. However, *Chlamydia* sp. was isolated from the eyes of two deer, a juvenile male and an adult female. Two deer were suspect on fluorescent antibody testing for IBR antigens; however no IBR viruses were isolated from any of the seven deer. No virus-like particles were observed by electron microscopy from the four deer tested. Serum immunodiffusion tests for antibodies to caprine arthritis-encephalitis/ovine progressive pneumonia were all negative.

DISCUSSION

Our results were consistent with smaller observational studies reported in the literature with the exception that we were able to document the clinical recovery of deer and we were able to identify etiological agents. In addition, *Moraxella* sp. was recovered from the eye surface of a clinically unaffected deer 1 yr after the epizootic occurred. Blindness was reported in 10 male mule deer in the Medicine Bow National Forest, Wyoming, during the spring of 1943 (Rosenfeld and Beath, 1944). Affected deer had reddish to opaque eyes, corneal ulceration, and purulent ocular exudate with the condition progressing to complete blindness. Unfortunately, no etiological agent was identified. Many cattle located nearby were reported to show similar, but not as severe clinical signs. Honess and Winter (1956) also reported that young antlered male mule deer ranging in the upper North Platte River drainage, Wyoming had vascular keratitis annually from 1943 to 1955; however no diagnostic information was reported.

Thelazia californiensis was reported in deer inhabiting Sequoia and Yosemite National Parks, California (USA) during 1934 (Rosenfeld and Beath, 1944). These animals had severe ocular inflammation, which produced temporary blindness but they clinically recovered within 2 to 3 wk.

The Zion Park staff was concerned that as the mule deer dispersed in the spring from Zion Canyon, the infection might spread into the nearby herd of desert bighorn sheep (*O. canadensis nelsoni*) which had been reintroduced. During the winter of 1981 to 1982, an epizootic of IK occurred in Rocky Mountain bighorn sheep in Yellowstone National Park (USA). Indirect mortality was estimated to be 60% of the herd (Meagher et al., 1992). However, as of July 1994, none of the desert bighorn sheep in Zion National Park have been observed with clinical signs of IK nor has significant mortality occurred.

The epizootic of infectious keratoconjunctivitis in mule deer at Zion National Park did not follow the trends that commonly occur in domestic livestock. The epizootic occurred during the cooler months of November through March, when fly populations were not active and during a period when hours of sunlight were decreased. No epizootics of IK in domestic livestock in the Zion area were reported Utah's Department of Wildlife Resources or Department of Livestock.

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