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Preliminary Studies on the Etiology of Keratoconjunctivitis in Reindeer (*Rangifer tarandus tarandus*) Calves in Alaska

Alina L. Evans,[1,5] Russell F. Bey,[1] James V. Schoster,[2] James E. Gaarder,[3] and Gregory L. Finstad[4]
[1] Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, 1971 Commonwealth Ave., St. Paul, Minnesota 55108, USA; [2] Animal Eye Consultants of Minnesota, Roseville, Minnesota 55113, USA; [3] Veterinary Eye Specialists, 1921 W Diamond Blvd., Suite 108, Anchorage, Alaska, 99515, USA; [4] Reindeer Research Program, University of Alaska, PO Box 757200, Fairbanks, Alaska, 99775; [5] Corresponding author (email: alinaevans@gmail.com)

ABSTRACT: Keratoconjunctivitis outbreaks occur each summer in reindeer (*Rangifer tarandus tarandus*) herds in western Alaska, USA. This condition has not been well characterized nor has a definitive primary etiologic agent been identified. We evaluated the eyes of 660 calves near Nome, Alaska, between 29 June and 14 July 2005. Clinical signs of keratoconjunctivitis were observed in 26/660 calves (3.9%). Samples were collected from the conjunctival sac of both affected ($n=22$) and unaffected ($n=24$) animals for bacterial culture, enzyme-linked immunosorbent assay testing for *Chlamydophila psittaci*, and for polymerase chain reaction assays for *Mycoplasma* and *Moraxella* spp. No primary bacterial or viral etiologic agent(s) were isolated or identified. The cause of keratoconjunctivitis among reindeer calves was not determined, but it could involve an anaerobic bacteria, a difficult-to-isolate viral agent, stress associated with repeated handling, ocular foreign bodies, exposure to corral dust or arthropods, or a combination.

Key words: Conjunctivitis, keratitis, keratoconjunctivitis, ocular disease, pink eye, *Rangifer tarandus tarandus*, reindeer, Seward Peninsula.

Semidomesticated reindeer (*Rangifer tarandus tarandus*) from Siberia were first imported to Alaska, USA, in 1891 to serve as a food and income source for the Yup'ik and Inupiaq peoples. In Alaska, reindeer are allowed to forage freely and are periodically rounded up with helicopters for slaughter, antler harvesting, and medical care (Dieterich, 1981).

Alaskan reindeer herders have requested assistance from state and federal agencies, including the University of Alaska Fairbanks, to identify the factors responsible for decreasing productivity. One potential loss of productivity is keratoconjunctivitis in calves, a painful and possibly contagious eye disease that can leave animals blind or with impaired vision. Keratoconjunctivitis is seen annually during the summer reindeer handlings on the Seward Peninsula (Reindeer Research Program, University of Alaska Fairbanks, unpubl. data).

Infectious keratoconjunctivitis has been studied in numerous other species. In cattle, the primary pathogen has been identified to be the piliated form of *Moraxella bovis* (Ruehl et al., 1988). *Moraxella ovis* has been implicated in infectious keratoconjunctivitis in moose (*Alces alces*), mule deer (*Odcoileus hemionus*), and goats (*Capra hircus*; Dubay et al., 2000). *Chlamydophila psittaci* has also been isolated from infected eyes in mule deer (Taylor et al., 1996) and Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*; Meagher et al., 1992). *Mycoplasma conjunctivae* has been isolated from infectious keratoconjunctivitis in sheep (*Ovis aries*; Akerstad and Hafshagen, 2004) and goats (ter Laak, 1988), alpine chamois (*Rupicapra rupicapra*; Tschopp et al., 2005), and ibex (*Capra ibex ibex*; Mayer et al., 1996). In addition, a variety of opportunistic bacteria have been isolated from reindeer (Aschfalk et al., 2003).

A primary etiologic agent causing keratoconjunctivitis in reindeer has not been identified. Keratoconjunctivitis is reported in reindeer throughout Finland and Scandinavia, and in many other species. Hadwen and Palmer (1922) and Dietrich (1981) have previously discussed keratitis as a clinical condition in Alaskan reindeer. To our knowledge, this is the first case

report of keratoconjunctivitis in Alaskan reindeer and the only report of keratoconjunctivitis that is limited to reindeer calves.

Reindeer (2,300–3,000) were herded by helicopter into a corral about 8 km north of Nome, Alaska. Sex was determined, and calves were tagged and weighed on a platform mounted on a Tru-test livestock scale (Pearson Livestock Equipment, Thedford, Nebraska, USA). Six hundred sixty calves, approximately 4–8 wk of age, were evaluated for keratoconjunctivitis on 29 June, 2 July, and 14 July 2005. Each animal's eyes were examined macroscopically, with the aid of a flashlight, for clinical signs of keratoconjunctivitis, including conjunctival inflammation, excessive lacrimation, squinting, corneal opacity and/or corneal ulceration. Data were collected on the presence of clinical signs, sex and body weight. If handled more than once, the mean weight was used. Affected animals were sampled with the next healthy animal sampled as a control. Swab samples were taken from the lower conjunctival sac of each animal using BBL Culture Swabs (Becton Dickinson, Sparks, Maryland, USA). Swab samples were streaked onto sheep blood agar plates and then stored in a culture medium composed of a mixture of brain heart infusion broth (Difco Laboratories, Detroit, Michigan, USA), 50% sheep blood, and 5% glycerin. Swab samples and blood agar plates were shipped on ice to the Washington Animal Disease Diagnostic Laboratory (WADDL) in Pullman, Washington, USA, for bacterial culture (blood agar plates), virus isolation (swabs), and enzyme-linked immunosorbent assay (ELISA) testing for *C. psittaci* (QuickVue ELISA test, Quidel Corporation, San Diego, California, USA; swabs). Sheep blood agar plates that could not be shipped to WADDL immediately were incubated at 37 C for 24 hr and then refrigerated. Swabs in the modified transport media were refrigerated until shipment. Samples reached WADDL within 24–72 hr of collection. All samples were subjected to routine bacteriologic testing as described by Quinn et al. (1994). Swabs were inoculated into bovine turbinate cells and Madin-Darby canine kidney and Crandall feline kidney cells. Cell cultures were incubated at 37 C, and if cytopathic effect was not observed after three passages at 4-day intervals, the sample was classified as negative for virus.

After testing was completed at WADDL, swabs were shipped to Kansas State University, Manhattan, Kansas, USA, for polymerase chain reaction (PCR) testing for *Mycoplasma* spp. and *M. bovis*. The PCR test for *Mycoplasma* spp. was performed as described by Lauerman (1998). The *M. bovis* PCR was done by randomly amplified polymorphic DNA (RAPD) PCR, using two different sets of primers. The resulting banding patterns were compared with the profiles of the American Type Culture Collection (Manassas, Virginia, USA) strain of *M. bovis*.

The eyes of 26 reindeer slaughtered during February and March 2006 were also examined. All of these animals were normal in appearance.

Of the 660 calves examined, 329 were males and 330 were females; sex was not recorded for one animal. Keratoconjunctivitis, based on any one of the following clinical signs was observed in 26 (3.9%) animals. Observed clinical signs included conjunctival inflammation, corneal neovascularization (3–5 mm), excessive lacrimation, squinting, corneal opacity, corneal ulceration, and/or blepharospasm. Eighteen of 26 (69.2%) affected animals exhibited severe disease, including both conjunctival and corneal signs. Twenty-two affected animals and 24 controls were sampled. There were no ocular parasites observed in cases or controls.

The average weight of all affected weighed animals was 26.4 kg ($n=23$, SD=3.6). The average weight of unaffected animals was 27.1 kg ($n=546$, SD=4.6); based on a two-sample *t*-test (Freedman et

al., 2007), affected animals weighed significantly less than the unaffected animals ($t=2.33$, $P<0.05$).

No pathogenic bacteria were grown on aerobic culture. A mixed population of non–β-hemolytic bacteria, including *Staphylococcus* spp., *Streptococcus* spp., and coliform bacteria, was isolated from affected and control animals. These bacteria are considered of low virulence and are commonly isolated from the environment and other nonsterile sites. No viruses were detected and all samples were negative for *C. psittaci*, *Mycoplasma* spp., and *M. bovis*.

Although there was a significant association between decreased weight and increased incidence of keratoconjunctivitis, a causal relationship was not confirmed. The observed blepharospasm indicates that the condition is most likely painful and neovascularization indicates that the disease was present for several days before examination (Martin, 2005). Because calves had been in the corral for 12–36 hr, these clinical signs indicate that the cause was present before handling.

A negative ELISA test (sensitivity 97.5%) for *Chlamydophla* strongly suggests that this organism is not involved in the etiology of infectious keratoconjunctivitis in Alaskan reindeer. Negative virus isolation results, however, do not rule out viral involvement in this disease as some potential viruses' capable of causing keratoconjunctivitis in cervids or ruminants may not readily grow in cell culture or may have been inactivated during transport.

The failure of bacterial culture to detect a primary pathogen indicates that the etiologic agent is most likely not bacterial or is an anaerobic or highly fastidious bacterial agent. Our methods were designed for detection of species such as *M. ovis*, *M. bovis*, *Branhammella ovis*, and the negative culture results indicate that these species are not involved because they are relatively easy to isolate and identify (Quinn et al., 1994). The PCR methods used for *Mycoplasma* spp. at the Kansas State University Veterinary Diagnostic Laboratory were shown to detect and differentiate between 11 bovine *Mycoplasma* spp. (Baird et al., 1999). Based on these results it seems that *Mycoplasma* spp. are not involved in keratoconjuctivitis in Alaskan reindeer calves.

The negative PCR results for *M. bovis* were inconclusive because the RAPD PCR works poorly in mixed cultures, and interpretation amplification patterns containing DNA from multiple organisms is difficult. However, we can reasonably rule out *M. bovis* as a primary etiologic agent based on the negative culture results in all 22 affected samples.

Bovine herpes virus 1 (BHV-1) or another herpesvirus with similar antigenicity has been found in Alaskan reindeer (Dieterich, 1981). In Finland, 23% of 300 domestic reindeer had serum neutralization antibodies to BHV-1, but cervid herpes virus 2 was isolated (Ek-Kommonen et al., 1986). An outbreak of ocular disease in red deer (*Cervus elaphus*) calves associated with a virus antigenically similar BHV-1 was reported by Inglis et al. (1983). In this outbreak, conjunctivitis, purulent ocular discharge, corneal opacity, and excessive lacrimation were observed in 50 to 60 of 80 calves. Of the 34 initial cases, 19 exhibited bilateral ocular signs (Inglis et al., 1983). Of our 26 cases, only one exhibited bilateral keratoconjunctivitis. It is possible that reindeer affected with a herpesvirus or other agent that causes bilateral lesions do not survive, but at present, herpes viruses have not been shown to cause keratoconjunctivitis in reindeer.

The impact of keratoconjunctivitis on reindeer calf survival is unknown and would be difficult to assess because reindeer are not consistently handled. In addition, the role of natural predation is unknown as is the normal survival rate.

Our data indicate that approximately 4% of reindeer calves 4–8 wk of age were affected with clinical signs of keratocon-

junctivitis. We noted that there were very few adults with corneal changes, suggesting that affected animals either die, as reported by Aschfalk et al. (2003), or recover completely.

Studies need to be conducted to better define this clinical condition in reindeer. We recommend establishing a case-fatality rate by comparing the yearly survival of affected and nonaffected calves. Conjunctival cytology may help determine the type of inflammation present and could direct diagnostic efforts towards select categories of pathogens.

Identifying an etiologic agent and risk factors with infectious keratoconjunctivitis will enable herders to take appropriate steps for managing the disease. More knowledge of this disease could lead to the development of treatments and preventative measures, thus improving reindeer health, limiting animal suffering, and increasing production.

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