Infectious keratoconjunctivitis in red deer (*Cervus elaphus*) in Poland – A case report

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Abstract: Infectious keratoconjunctivitis (IKC) has not been observed in European deer *(Cervus elaphus)*. Our case concerned two red deer bulls in a natural environment, which had orientation disorders and/or circle movement. A detailed post-mortem examination of one of the bulls revealed extensive conjunctivitis in both eyes, clouding and ulceration of the cornea. There were no lesions in the other organs, particularly in the central nervous system (CNS). The histopathological examination of the eyeballs showed corneal epithelium erosions and vascularisation and an inflammatory infiltration of the stroma. Descemet's membrane was found to be disrupted and the corneal stroma was fused with the iris. The remaining structure of the eye did not show any lesions. Furthermore, the polymerase chain reaction analysis for OvHV-1, BHV-1, BHV-5, CapHV-1, CcHV-1, and EHV-1 showed negative results. In the agar culture, only single bacteria were present. There were no *Thelazia* spp. worms in the conjunctival sac, but numerous *Lipoptena cervi* flies were present on the skin in the eye region and on the corneal surface. The findings of our case study and those reported in the literature suggest that IKC is a polyetiological disease, where the composition of the pathogenic agents may differ from case to case. The present study suggests that the mechanical irritation of *L. cervi* parasites could be a contributing factor to the corneal damage, infection and chronic inflammation resulting in a loss of vision in red deer.

Keywords: herpesvirus; *Lipoptena cervi*; ocular infections; wildlife

Infectious keratoconjunctivitis (IKC) is an ocular disease affecting cattle and sheep worldwide (Dagnall 1994; Brown et al. 1998). The disease often starts with conjunctivitis, lachrymation, and a corneal oedema. If it does not heal spontaneously, the conjunctivitis intensifies – a strong oedema occurs with ulceration of the cornea. In extreme cases, this process can lead to panophthalmitis. IKC cases have also been reported in the free-ranging deer. In 1922,

Hadwen and Palmer observed keratitis in reindeer (*Rangifer tarandus*) in Alaska (Hadwen and Palmer 1922) and similar observations were also reported by Dietrich (1981), Loison et al. (1996), and Taylor et al. (1996). In the last decade, numerous studies have been reported on IKC in reindeer (Evans et al. 2008) and mule deer (*Odocoileus hemionus*) in the western states of the USA (Dubay et al. 2000; Edmunds et al. 2008; Munoz Gutierrez et al. 2018);

however, the aetiopathogenesis of the disease is still not clearly understood. Some of the aetiologic agents of IKC reported in the literature include *Moraxella bovis* (Hughes-Pugh 1970; Ruehl et al. 1988) isolated from cattle, *Moraxella ovis* (Dubay et al. 2000), *Chlamydia psittaci,* and *Mycoplasma conjunctivae* (Dagnall 1994; Akerstad-Ofshagen 2004) isolated from sheep. In addition, *Mycoplasma conjunctivae* (Laak et al. 1988; Tschopp et al. 2005) or *Mycoplasma ovis* (Dubay et al. 2000) were isolated from goats and chamois, and *Moraxella* spp. and *Chlamydia psittaci* (Taylor et al. 1996) from mule deer cases. Evans carried out a study on a sample of 660 reindeer calves, and the results showed the presence of keratoconjunctivitis symptoms in 3.9% of the examined calves. However, the results of the bacteriological tests, cell culture inoculation, polymerase chain reaction (PCR) tests, and enzyme-linked immunosorbent assay (ELISA) tests did not show any microbiological pathogenic factors (Evans et al. 2008). In addition, Edmunds reported keratoconjunctivitis and panophthalmitis in mule deer caused by *Yersinia pestis* (Edmunds et al. 2008). Dietrich's study on viral agents associated with IKC in reindeer showed the presence of the BHV-1 virus (Dietrich 1981). The disease was also reported to be observed in moose, capricorn, roe, and red deer (Giacometti et al. 2002; Gupta et al. 2015). To date, there have been no reports related to IKC in free-ranging animals in the Polish literature.

Case description

In September and November 2017, two red deer bulls (*Cervus elaphus*) from the area of the Oborniki Śląskie forest district (west Poland) were selected for the present study, which had a circular movement and spatial orientation disorder. One of them – an irregular twelve pointer bull with a good body condition, in the daytime with a human presence, could run for approximately half an hour in a cut forest. The animal was making circular movements and falling into tree branches. At the beginning of November, at a distance of seven kilometres from the previous observation site, another animal showed a similar behaviour. The animal was an irregular fourteen-pointed bull weighing 120–130 kg. On the meadow in the forest, the animal was making circular movements in a 2–3 m radius. Later, at the end of December, this bull with signs

of strong emaciation was shot dead. The head and internal organs were subjected to a morphological examination.

The eyeballs and conjunctival sack's fragments were fixed in formalin. Histopathological slides were prepared by haematoxylin and eosin (H&E) staining.

In the study, we used a nested-PCR to detect and identify ovine herpesvirus 2 (OvHV-2) from the sample collected on a swab from the conjunctiva of the red deer. The DNA was isolated using a DNA Mini Kit (A&A Biotechnology, Gdynia, Poland). The PCR analysis was performed with the following primers:

- 556 primer (5'-AGTCTGGGGTATATGATC CAGATGGCTCTC-3').
- 775 primer (5'-AAGATAAGCACCAGTTAT GCATCTGATAAA-3').
- 555 primer (5'-TTCTGGGGTAGTGGCGAG CGAAGGCTTC-3').

In the first step, primers 556 and 775 were used to obtain a PCR product of 422 bp. In the second step, primers 556 and 555 were used to obtain a PCR product of 238 bp. For the PCR analysis, 10 pmol of each primer, a 10× DreamTaq Buffer, 0.5 µl of dNTPs, 1 µg of DNA, a DreamTaq DNA Polymerase 1.25 IU, and nuclease-free water to a final volume of 25 µl were added to the sample. The thermal cycling conditions for both steps were 5 min at 94 °C, then 40 cycles of 94 °C for 30 s and 72 °C for 90 s, and then a final extension step at 72 °C for 10 minutes. After amplification, the reaction mixture was electrophoresed on a 2% agarose gel and visualised using an ultraviolet (UV) transilluminator.

The PCR was performed according to the method of Ababneh et al. (2014). The swab was also inoculated into a blood agar and incubated at 37 °C for 24 hours.

The detailed morphological examination only showed fermentation disorders in the rumen and numerous medium-sized pulmonary worm cysts. In addition, the autopsy and histopathological examination of the brain did not show any abnormalities, but, in the eyes, a strong inflammation of the conjunctiva was noted, with congestion, tear exudation, and the presence of bilateral corneal opacity. The opacity was the strongest in the centre of the cornea. In the right eye, a corneal ulceration and perforation were detected. Numerous *Lipoptena cervi* flies were found on the bull's skin. One of these parasites was also present on the right eye's

cornea. In the conjunctival sac, no *Thelazia* spp. worms were present.

In the microscopic examination, the squamous epithelium of the cornea showed varied thicknesses – there were regions with 12 and 8 cell layers. In some areas, the epithelium underwent damage, sometimes including basement membrane damage. In the corneal stroma, bunches of collagen fibres were often swollen and stratified. In the corneal limbus region, vascularisation of the stroma, numerous medium-sized cellular infiltrates, often in a linear shape, and a few pigment cells were detected. Descemet's membrane on most of examined areas were well visible, locally swollen, and the endothelium was well preserved. However, in the central region of the cornea, Descemet's membrane was found to be damaged, and the stroma was permanently fused with the iris with massive vascularisation and lymphocyte and histiocyte infiltration. The iris vessels were dilated and filled with erythrocytes. The ciliary body, lens, and other structures of the eyeball were found with no important abnormalities. In the third eyelid, congestion and a small inflammatory infiltration were observed. Moreover, the result of the PCR analysis was negative. Only a few colonies of *Proteus* spp. and *E. coli* were observed on the blood agar.

DISCUSSION AND CONCLUSIONS

Previous reports on the IKC aetiology have shown that the pathogenic factors may vary considerably from case to case and that they partly depend on the animal species. The aetiologic agents most often reported in the literature include *Moraxella* spp., *Chlamydia* spp., and *Mycoplasma* spp. Dietrich (1981) reported the presence of a BHV-1 virus associated with IKC in reindeer. Neves et al. (2010) reported the presence of a CvHV-2 herpesvirus in Norwegian reindeer with IKC symptoms. Tryland et al. (2009) reported that the primary pathological changes in reindeer calves were induced by a CvHV-2 virus and later complicated by *Moraxella bovoculi*. In a sample of twenty-six calves with evident symptoms of keratoconjunctivitis, Evans et al. (2008) did not isolate any pathological bacteria or viruses. The role of the deer's parasites also seems to be interesting. The *Thelazia* species-common nematodes of a mule deer's conjunctival sac do not cause a noticeable inflammation. In deer from Central Europe,

Figure 1. Complete corneal clouding with central ulceration. *Lipoptena cervi* on the skin close to eye angle and on the corneal surface

the deer ked (*L. cervi*) seem to play an important role in the development of keratoconjunctivitis. In late summer, a massive population of *L. cervi* starts to appear in the deer area. Their presence in the areas of the deer's eyes and on the corneal surface is not random because in red deer, preorbital fragrances exist in these areas (Figure 1). Their activity is particularly high in the rutting period. The ked (*L. cervi*) have the ability of flight, and fragrance stimuli help them to find a host. When they settle in the eye region, they can transfer the bacterial or viral agent and also directly damage the corneal surface, thus, making the eye more susceptible to infections.

To conclude, IKC in deer is not an integral disease entity. It can be caused by various pathogenic factors with a varying extent. Hence, in some IKC cases, the primary initiating factor can be the mechanical damage of the corneal surface or the conjunctival mucosa by mechanical factors, such as strong air pollination or parasitic arthropods. In other IKC cases, the disease is initiated by viral infections. In both cases, a secondary bacterial infection caused by *Moraxella* spp., *Chlamydia* spp., or *Mycoplasma* spp. can develop rapidly.

Conflict of interest

The authors declare no conflict of interest.

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