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Source: Journal of Wildlife Diseases, 45(2) : 508-511

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.2.508

Journal of Wildlife Diseases, 45(2), 2009, pp. 508–511

Investigation of the Role of Austrian Ruminant Wildlife in the Epidemiology of Malignant Catarrhal Fever Viruses

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ABSTRACT: Malignant catarrhal fever (MCF) is an ubiquitous disease of cattle and other ruminants caused by *Ovine herpesvirus 2* (OvHV-2), which is endemic in sheep and transmitted from healthy carriers. Further viruses of the MCF group are also able to induce MCF in ruminants. As alpine pasturing is very common in Austria, possible contact with ruminant wildlife carrying and excreting MCF viruses might be suspected as an infection source. To investigate the epidemiologic role of Austrian deer and chamois, spleen samples were collected from 55 red deer (*Cervus elaphus*), 72 roe deer (*Capreolus capreolus*), four fallow deer (*Dama dama*), and five chamois (*Rupicapra rupicapra*) during the hunting seasons 2001–2003. Samples were tested by both herpesvirus consensus and OvHV-2–specific polymerase chain reaction. As all spleen samples tested negative, there is no indication that in the region and period investigated, MCF viruses circulated in wild ruminants.

Key words: Austria, deer, epidemiology, herpesvirus, MCF, OvHV-2.

Malignant catarrhal fever (MCF) is a severe often fatal disease of cattle and other ruminants characterized by lymphoproliferation of blood and lymph vessels and mucosal surfaces. The clinical disease had been related to direct or indirect contact to asymptomatic sheep (sheep associated [SA]-MCF, worldwide) or wildebeest (wildebeest associated [WA]-MCF, Africa and zoological gardens). The causative agent of SA-MCF, *Ovine herpesvirus 2* (OvHV-2) is a member of the *Herpesviridae* subfamily *Gammaherpesvirinae*, genus *Macavirus*. The epidemiology of SA-MCF is not yet completely clarified. OvHV-2 is endemic in sheep and excreted by healthy carriers (Imai et al., 2001). In adult sheep OvHV-2, was found in lymphocytes, the probable site of latent infection (Baxter et al., 1997). Although direct or indirect contact with sheep has been documented in most cases of MCF, horizontal transmission in cattle or the existence of other ruminant reservoirs cannot be excluded. Malignant catarrhal fever in deer has been long known, has worldwide distribution, and causes important economic losses in farmed deer (Huck et al., 1961; Clark and Robinson, 1970; Sanford and Little, 1977).

Clinical symptoms in deer are described as follows: conjunctivitis; mucopurulent rhinitis; diarrhea; skin lesions around the nose, mouth, eyes, anus, and feet; mucosal ulcers on lips and tongue; enlargement of several lymph nodes; and severe central nervous system symptoms (ataxia, torticollis, incoordination). The course of the disease is also often peracute or characterized by sudden onset of clinical symptoms shortly before death (Sanford and Little, 1977; Haigh et al., 2002; Keel et al., 2003). As causative agents, different gammaherpesviruses of the MCF group have been found in the highly susceptible deer: *Alcelaphine herpesvirus 1* (WA-MCF; Castro et al., 1984; Heuschele et al., 1985), OvHV-2 (Tham, 1997; Crawford et al., 1999), and another closely related gammaherpesvirus, *Caprine herpesvirus 2* (CpHV-2), possibly endemic in goats (Chmielewicz et al., 2001; Li et al., 2001; Crawford et al., 2002; Keel et al., 2003). Recently, CpHV-2 and OvHV-2 DNA were detected during a retrospective study in Norway (1982–2005) in organ samples

of free-ranging moose (*Alces alces*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*) with typical MCF lesions (Vikoren et al., 2006). A new gammaherpesvirus causing MCF in deer was described by Li et al. (2000) and Kleiboeker et al. (2002) in white-tailed deer (*Odocoileus virginianus*).

There are no data available about the prevalence of gammaherpesviruses of the MCF group in particular of OvHV-2 in Austria. Antibody prevalences of 11% in free-ranging fallow deer (*Cervus dama*) and of 0% in roe and red deer were reported from Germany (Froelich et al., 1998) and all antibody-positive samples originated from one circumscribed area in Northern Germany (Schleswig-Holstein). Nevertheless Froelich et al. (1998) did not detect OvHV-2 in spleen or lymph nodes of deer, this including antibody-positive animals.

In clinical cases of MCF, a variety of organs (spleen, lymph nodes, liver, intestine, brain, lung, and kidney) have been shown to be suitable for the detection of OvHV-2 (Crawford et al., 1999; Dunowska et al., 2001).

Local practitioners in Austria reported outbreaks of MCF in cattle herds without any known direct or indirect contact with sheep; in some cases these outbreaks were epidemic. The practice of alpine pasturing of cattle and sheep herds during the summer allows indirect and direct contact of both species with wild ruminants, in particular deer and chamois.

In the course of a previous study (Krametter et al., 2004), spleen samples of deer and chamois killed during the hunting seasons 2001–2003 had been collected. These samples were now further investigated for both the presence of OvHV-2–specific as well as of herpesviral DNA in search for further ruminant reservoirs of the virus for domestic cattle and sheep.

Spleen samples were collected during the hunting seasons of 2001–2003 from 55 red deer, 72 roe deer, four fallow deer (*Dama dama*), and five chamois (*Rupicapra rupicapra*) from nine hunting districts ($n=127$) and one deer farm ($n=9$) in northeast Carinthia in the south of Austria (46°33′–47°N, 14°30′–14°75′E).

Hunters recorded species, age, sex, and weight of each animal. All 136 animals were apparently clinically healthy. Spleen samples (approximately 30–50 g/animal) were immediately transferred to refrigerated containers and sent to the laboratory. When not processed immediately, spleen samples were stored at −80 C. Approximately 10 mg of tissue (collected from two areas of the pulp) were lysed by Proteinase K digestion (Promega; in buffer ATL, Qiagen, Austria). Nucleic acids were extracted using a commercially available kit (QIAamp Viral RNA Kit, Qiagen, Hilden, Germany).

For the detection of the OvHV-2 tegument protein gene, polymerase chain reaction (PCR) was carried out as described by Schenz et al. (2000). Additionally, pooled extracts (three samples/pool) were tested with consensus-nested PCR with the use of degenerate primer sets targeting the DNA–polymerase gene of several γ, α, and β herpesviruses, human and animal (VanDevanter et al., 1996). Negative and positive controls for DNA extraction and PCR were run with every assay.

Extracts of amplified DNA (NucleoSpin®Extract, Machery-Nagel, Düren, Germany) served as template for sequencing PCR (DNA Sequencing Kit, Applied Biosystems, Warrington, UK). Sequencing PCR products were analyzed with the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Norwalk, Connecticut, USA).

In the PCR specific for OvHV-2 (Schenz et al., 2000), 134/136 samples tested negative. Two samples gave a questionable result but by sequence analysis both amplification products were shown to be nonspecific. By herpesvirus consensus PCR, all samples tested negative.

A sample collection representative for

regional ruminant wildlife (Carinthia in Southern Austria) was available. The study area has a deer density of 0.145 deer/ha (0.035 red deer and 0.11 roe deer) of cultivated area. Chamois and fallow deer densities are much lower than the red deer density in this area. All killed animals were apparently healthy. All spleen samples investigated tested negative for OvHV-2. These results are in accordance with Froelich et al. (1998) who found no OvHV-2 positive samples (spleen or lymph nodes) in free-ranging deer from Germany.

By herpesvirus consensus nested PCR with the use of degenerate primers targeting a conserved region of the DNA polymerase gene (VanDevanter et al., 1996), all samples tested negative. Therefore, there is no indication of additional viruses of the MCF group circulating in roe and red deer of the region investigated. This is in contrast to Canada and the USA, where CpHV-2 and other closely related MCF viruses are described in different ruminant species (Crawford et al., 2002; Keel et al., 2003; Li et al., 2003).

In the study by Froelich et al. (1998), only fallow deer tested antibody positive (11% of animals tested) and furthermore all of these animals originated from one circumscribed area in Northern Germany (Schleswig-Holstein). In our study, blood samples were not available and antibody prevalence against viruses of the MCF group could not be estimated; however, no evidence of infection was detected with the use of PCR.

With regard to MCF cases in cattle without any known contact to sheep, wildlife ruminants have been suggested as a possible source of infection during alpine pasture. The results of our study do not suggest an epidemiologic importance of roe and red deer for the spreading of OvHV-2, CpHV-2, or other gammaherpesviruses; however, the number of chamois and fallow deer was too low to allow for any definitive conclusions. Additional work, including antibody testing, is needed to understand the potential for wildlife involvement in MCF transmission in this area.

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Received for publication 19 November 2007.