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Source: Journal of Wildlife Diseases, 40(4) : 791-795

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.4.791

Journal of Wildlife Diseases, 40(4), 2004, pp. 791–795

Pestivirus Exposure in Free-living and Captive Deer in Austria

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ABSTRACT: During the hunting season of 2001–02, blood and spleen samples from 59 red deer (*Cervus elaphus*), 77 roe deer (*Capreolus capreolus*), four fallow deer (*Dama dama*), and five chamois (*Rupicapra rupicapra*) were collected from nine hunting districts ($n=133$) and one deer farm ($n=12$) in southern Austria. Sera were tested for antibodies against bovine viral diarrhea virus (BVDV) with an enzyme-linked immunosorbent assay (ELISA) and virus neutralization tests against three BVDVs and one border disease virus strain. Reverse transcriptase polymerase chain reaction was used for detection of pestivirus-specific RNA in spleen samples. Antibodies were detected in one serum sample when using ELISA and virus neutralization tests. Results of the virus neutralization tests of this sample provided strong evidence for the exposure to the BVDV-1 genotype. The spleen samples were negative for pestivirus-specific RNA.

Key words: Bovine viral diarrhea virus, border disease virus, free-living deer, serologic survey, reverse transcriptase polymerase chain reaction.

The genus *Pestivirus* in the family *Flaviviridae* comprises bovine viral diarrhea virus (BVDV), border disease virus (BDV), and classical swine fever virus (Horzinek, 1991). Common hosts of ruminant pestiviruses are livestock such as cattle and sheep (Loken, 1995). Surveys of cattle sera from several countries showed that about 70% of the animals had serum neutralizing antibodies against BVDV (Houe, 1995). Pestivirus antibodies in sheep have been detected in several countries, but usually the prevalence is much lower than in cattle (Nettleton and Enterican, 1995).

Apart from cattle and sheep, pestiviruses have been isolated from many other ruminant species including red deer (*Cervus elaphus*) (Nettleton et al., 1980), roe deer (*Capreolus capreolus*) (Schellner, 1977; Frölich and Hofmann, 1995), fallow deer (*Dama dama*) (Neumann et al., 1980; Weber et al., 1982; Diaz et al., 1988), and mule deer (*Odocoileus hemionus*) (Van Campen et al., 2001). Frölich and Hofmann (1995) isolated cytopathogenic pestiviruses from the spleens of two seronegative, free-ranging roe deer. Sequence analysis placed these isolates in BVDV genotype 1 (BVDV-1), but subgrouping was not feasible (Fischer et al., 1998). Antibodies reactive to ruminant pestiviruses have been found in red deer (Dedek et al., 1988; Schmitt and Wittkowski, 1999; Nielsen et al., 2000), roe deer (Dedek et al., 1988; Frölich, 1995; Schmitt and Wittkowski, 1999), mule deer (Couvillion et al., 1980), fallow deer (Frölich, 1995; Cuteri et al., 1999), and caribou (*Rangifer tarandus caribou*) (Elazhary et al., 1981).

The epidemiology of pestivirus infection in free-living ruminants is not well known. Cattle persistently infected (PI) play a key role in the epidemiology of infection among domestic ruminants (Loken, 1995). However, the importance of PI cattle and sheep as a source of infection for free-living deer needs to be clarified. There remains the possibility of transmission to susceptible domestic ruminants from free-living ruminants because the virus has been isolated from free-ranging deer (Nettleton et al., 1980; Frölich and Hofmann, 1995; Van Campen et al., 2001). According to Loken (1995), wild animals might occasionally introduce the virus to susceptible domestic animals on pasture, for example, by chance contact or licking the same salt stones.

The objectives of this study were to determine the seroprevalence of BVDV and BDV in free-living and captive deer in

southern Austria and to determine the frequency of pestiviral infections in these animals.

The study area, northeast Carinthia in southern Austria (46°33′ to 47°N, 14°30′ to 14°75′E), has a cattle density of 0.8 per hectare and a sheep density of 0.1 per hectare of cultivated area (Statistik Austria, unpubl. data). Alpine pasturing of cattle is centuries old in this area. This farming practice involves pasturing cows, heifers, and calves from different herds on alpine meadows from June to September. Based on information from local foresters and hunters, the deer density was 0.145 deer per ha (0.035 red deer per hectare and 0.11 roe deer per hectare) of cultivated and forest area. Chamois (*Rupicapra rupicapra*) and fallow deer densities are much lower than red deer density in this area.

Between May 2001 and December 2002, 145 blood and 145 spleen samples from 133 free-living animals (50 red deer, 77 roe deer, one fallow deer, and five chamois) and from 12 captive deer (nine red deer and three fallow deer) originating from a deer farm were collected by local hunters. Hunters collected blood from the heart, a major blood vessel, or from the thoracic cavity immediately after death, and estimated age of the animals based on dental wear. Coagulated blood and spleen samples, together with information indicating species, age, sex, weight, and origin of the blood sample, were sent to the laboratory in refrigerated containers. Among the animals studied were 22 male and 37 female red deer, 36 female and 41 male roe deer, four female and one male chamois, and three female and one male fallow deer, all aged between 1 yr and 18 yr. Conveyance by mail took between 1 day and 2 days. After centrifugation, serum samples were stored at −20 C, and spleen samples at −80 C.

Sera were tested at Steins Laboratory A/S (Brørup, Denmark) for antibodies against a BVDV-1 strain by using a blocking enzyme-linked immunosorbent assay (ELISA) (Roensholt et al., 1996). Three cytopathic BVDV strains (BVDV-1 strain NADL, BVDV-1 strain Oregon, and BVDV-2 strain "125") were used, as well as one noncytopathic BDV strain (BDV strain Chemnitz) for virus neutralization tests. Sera were heated at 56 C for 30 min before being tested for neutralizing antibodies, 50 μl of inactivated serum was diluted in twofold serial dilutions (1:2–1:2,048) prepared in 50 μl of Eagle's minimum essential medium, and inoculated in two rows per serum. Fifty microliters of a BVDV stock solution containing 100 50% tissue culture infective doses ($TCID_{50}$) were added to the serum dilutions. The mixtures were incubated for 60 min at 37 C in microtiter plates. Afterward, 50 μl of a cell suspension (3×10^5 cells/ml) of BVDV-free Madin Darby bovine kidney cells including 10% fetal calf serum were seeded into each well and the plates were sealed and incubated at 37 C for 3 days. Cell cultures were evaluated for cytopathic effects. Antibody titers were determined according to the methods of Spearman (1908) and Kärber (1931). Titers<1:4 were considered negative.

Spleen samples (10 mg) were cut into small pieces, placed in an Eppendorf tube, 180 μl of buffer ATL (Tissue lysis buffer, Qiagen, Valencia, California, USA) and 20 μl of Proteinase K (Qiagen) were added to the tissue, and then incubated at 56 C until complete lysis was achieved. Proteinase K was inactivated at 95 C for 8 min. A volume of 140 μl was placed in an Eppendorf tube and used for RNA extraction by using a kit (QIAmp Viral RNA Mini Kit, Qiagen). A panpestivirus polymerase chain reaction (PCR) was performed by using the primers published by Vilcek et al. (1994), as described by Dünser et al. (1999) by using a PCR kit (One Step RT-PCR Kit, Qiagen). The reverse transcriptase PCR (RT-PCR) used had been tested under the conditions provided with various sample materials (blood and organs including spleen, milk, and mucosal biopsies) with consistently positive results in PI cattle and in some transiently infected an-

imals. Compared to virus isolation, the RT-PCR detected 0.1 $TCID_{50}$ when using the BVDV strain NADL. At least one positive and one negative control were included in each assay.

Of 145 sera tested, only one sample (0.7%) from a 9-yr-old male red deer was positive by ELISA and virus neutralization. The serum had geometric mean antibody titers of 1:91 against BVDV-1 strain NADL, 1:32 against BVDV-1 strain Oregon, and 1:8 against BDV strain Chemnitz. The red deer sample did not have neutralizing activity against BVDV-2 strain "125" (titer<1:2). Spleen samples from all 145 animals were negative for pestivirus-specific RNA.

This is the first report of antibodies to ruminant pestiviruses in deer in Austria. One of 59 red deer had antibodies to BVDV-1 and BDV. The difference between the geometric mean titer of the two BVDV-1 strains and the BDV strain was 6.7-fold. This and the negative neutralizing result against BVDV-2 provide strong evidence that the deer was exposed to BVDV-1 (Wolfmeyer et al., 1997). Although the number of tested animals was small, the seroprevalence of 2% in red deer is close to the 3% prevalence measured in Bavaria (Schmitt and Wittkowski, 1999) and the 5% prevalence documented from Denmark (Nielsen et al., 2000).

In contrast to other reports (Frölich and Hofmann, 1995), we did not detect any infected deer by PCR, although an RT-PCR was used that detects a broad range of pestiviruses from pigs, cattle, and sheep (Vilcek et al., 1994) and that had been evaluated for various sample types.

The habitat of deer and cattle in our study area, aside from the lower cattle density, is similar to parts of Bavaria where Frölich (1995) described a cattle density of 1.5 cattle per hectare of cultivated area. Contrary to Schmitt and Wittkowski (1999), Frölich (1995) found a seroprevalence of 9% in red deer in Bavaria. In contrast to serologic surveys in Germany reporting BVDV-seropositive roe deer (Dedek et al., 1988; Frölich, 1995; Schmitt and Wittkowski, 1999), antibody-positive roe deer were not detected in Denmark (Nielsen et al., 2000) or in our study.

In our study area, the seroprevalence for BDV and BVDV in farmed sheep is 1.1% (Krametter, 2002). We estimate a BVDV seroprevalence among cattle of 70–80% and a prevalence of persistently viremic cattle of 0.5–1% in this area (Krametter, unpubl. data). These prevalences are similar to those documented by Houe (1995) for other countries. In this part of Austria, communal alpine pasturing of cattle during the summer is an important part of farming. Herders observed ruminant wildlife, predominantly red deer and chamois, grazing and licking salt stones on these Alpine pastures. The seropositive red deer was killed near such a communal alpine pasture.

The source of BVDV for free-living deer is still a subject of speculation. As in many regions, the seroprevalence in cattle is much higher than among free-living deer (Neumann et al., 1980; Schmitt and Wittkowski, 1999); infected cattle may play a role in the epidemiology of BVDV infection in free-living deer. The potential role of cattle as BVDV reservoirs for deer is supported by findings from Anticosti Island (Quebec, Canada), where deer have not had contact with cattle for 50 yr and all deer sera tested were negative for BVDV antibodies (Sadi et al., 1991). On the other hand, Frölich (1995) did not find significant differences in antibody prevalence among deer in regions with high, intermediate, or very low density of cattle. These results support the idea that free-living deer can become infected with BVDV without having contact with infected cattle. However, the management of wild and domesticated ruminants in our study area is much different than two of the regions described by Frölich (1995). Based on the low seroprevalence in deer, the fact that we did not identify any PI deer, and the high number of seropositive cattle in this area, we believe it more prob-

able that PI cattle transmit BVDV to red deer than vice versa. Nevertheless, the role and epidemiology of BVDV and BDV in roe and red deer herds in Austria still remain unclear and further investigations are needed.

The authors would like to thank R. A. Krametter for coordinating the sample collection and the involved local hunters for their cooperation in collecting the samples. We also would like to thank C. Pallan and M. Karin Walk for their excellent technical assistance.

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Received for publication 3 November 2003.