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Bovine Virus Diarrhea and Mucosal Disease in Free-ranging and Captive Deer (Cervidae) in Germany

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ABSTRACT: From 1990 until 1992, 355 blood samples of roe deer (*Capreolus capreolus*) (n = 123), red deer (*Cervus elaphus*) (n = 60), fallow deer (*Dama dama*) (n = 87) and other cervid species (n = 85) from three different habitats (n = 180) and 11 wildlife parks or zoos (n = 175) in Germany were tested for prevalence of pestivirus antibodies. Seventeen samples were seropositive for bovine viral diarrhea virus (BVDV); only one animal had antibodies for Border disease virus. Microneutralization test titers ranged from 1:5 to 1:125. We found no significant difference in antibody prevalence among deer in habitats with high, intermediate and low density of cattle. There were significantly more seropositive individuals in roe deer compared to fallow deer. Significantly more seropositive individuals were found among juvenile animals than among adults. Antibody prevalence in free-ranging cervids was significantly higher compared with that of deer in enclosures. Antibody prevalence in summer was significantly higher than in winter.

Key words: Epizootiology, bovine viral diarrhea virus, Border disease virus, *Capreolus capreolus*, *Cervus elaphus*, *Dama dama*, wildlife parks, cattle density.

Bovine virus diarrhea (BVD) and mucosal disease (MD) are generalized viral infections affecting a broad range of hosts including cervids (Schepers, 1990). The bovine viral diarrhea virus (BVDV) belongs to the genus *Pestivirus* within the Family Flaviviridae (Horzinek, 1990).

The natural mode of transmission of BVD and MD to cervids and the question of whether cervids could serve as a reservoir, is not yet clear (Thorsen and Henderson, 1971). Transmission in cattle is mainly by droplet infection between infected and susceptible animals (Schepers, 1990). Cattle density may be important because of the possibility that cattle contaminate common pastures of cattle and wild ungulates (Neumann et al., 1980). Experimental transmission of BVD and MD from domestic animals to cervids was demonstrated by Richards et al. (1956). However, transmission in these investigations was by intramuscular injection of infected organ material.

Clinical signs in cattle include transient, acute infections which may be inapparent or mild, or mucosal disease which is inevitably fatal (Brownlie, 1990).

Serologic surveys on BVD have been successfully conducted in a variety of deer species on many continents. In free-ranging deer, the highest seroprevalence was about 60% in Canadian caribou (*Rangifer caribou*) and was found by Elazhary et al. (1981). In the southern part of the former Federal Republic of Germany, Weber et al. (1978) found specific antibodies against BVDV in 6.6% of red deer and 5.9% of roe deer samples. In the former German Democratic Republic, however, only 0.6% of cervid sera was found to be seropositive (Dedek et al., 1988).

We were interested in learning whether cattle are a potential hazard for cervids and whether cervids represent a BVD reservoir for domestic animals. Our objective was to compare antibody prevalence to BVDV among cervids in habitats with high, intermediate and low cattle density; and between cervids in enclosures and free-ranging populations.

Hunters provided the blood samples together with information on prepared forms indicating species, age and sex. All animals were shot and blood was drawn immediately after death.

The samples originated from hunting areas in Schleswig-Holstein (54°30′ to 54°50′N, 9°20′ to 9°40′E), which have a cattle density of >2.6 cattle per ha of cultivated area; from forests in Berlin (52°30′N, 13°20′E), with a cattle density of

TABLE 1. Distribution of deer samples in different areas of Germany, November 1990 to October 1992.

	Roe deer	Fallow deer	Red deer	Exotic cervids
Schleswig-Holstein	56	32	6	—
Berlin	41	1	—	—
Bavaria	24	—	20	—
Wildlife parks	1	35	7	—
Berlin zoos	1	19	27	85

0.03 cattle per ha of forest area; and from hunting areas in Bavaria (48°00′ to 48°50′N, 9°30′E to 13°10′E), with a cattle density of 1.5 cattle per ha of cultivated area (Anonymous, 1987, 1990). The inclusion of the Berlin area was valuable due to its low cattle density and simultaneously high deer population density. Prevalence of BVD antibodies in cattle was 70 to 80% in all areas (Wizigmann, 1984). Roe deer (*Capreolus capreolus*) population density estimates have a high degree of uncertainty (Kurt, 1991). Based on questionnaires of local foresters, we believed that deer densities were similar in all three areas. Additionally, we evaluated serum samples from nine wildlife parks from Schleswig-Holstein and Bavaria and the two zoos in Berlin.

We collected 355 usable blood samples between November 1990 and October 1992. These samples were chiefly from roe deer (n = 123), fallow deer (*Dama dama*) (n = 87) and red deer (*Cervus elaphus*) (n = 60) (Table 1). Cervids up to 2 yr old (yearlings) and fawns were considered as juveniles; adult animals were over 2 yr old.

Coagulated blood was sent to the laboratory. Conveyance by mail took between 1 and 6 days. After centrifugation, serum samples were inactivated at 56 C for 30 min and stored at −20 C. Two different BVDV strains were used (Grub 313/83 and NADL), as well as one Border disease virus strain (cytopathic strain from the Moredun Institute in Edinburgh, United Kingdom), for neutralization tests.

A microneutralization test as described by Frey and Liess (1971), was used for the detection of pestivirus antibodies. All tests were performed in microtiter plates using 1000 $TCID_{50}$ (dose infecting 50% of the inoculated tissue culture cells) BVD or Border disease virus/ml and two-fold serum dilutions. The neutralization test was performed for 1 hr at 37 C. Subsequently, 3×10^5 cells/ml were seeded into each well. Georgia bovine kidney cells (American Type Culture Collection, Rockville, Maryland, USA) and sheep chorioid plexus cells (American Type Culture Collection) were used for verification of BVDV and Border disease virus, respectively. Three to 5 days later, the formalin-fixed cell cultures were evaluated for the presence of cytopathic effects (Frost et al., 1990). Antibody titers were calculated according to Spearman and Kärber (1985). Titers >1:4 were considered positive (Malmquist, 1968).

Fisher's exact test (Freeman and Halton, 1951) was used to evaluate differences in antibody prevalence between deer in habitats with different cattle density, adults and juveniles, males and females, free-ranging deer and deer in enclosures, deer shot in the summer and winter period, and different cervid species. The significance level was set at $\alpha = 0.05$. It should be noted that the statistical analysis must be regarded as exploratory, because multiple comparisons were based on the same data set.

Of 355 free-ranging and captive deer, 17 samples had antibodies against BVDV and only one positive reactor (an adult male roe deer) for Border disease virus was found. Fifteen of the 18 seropositive animals were roe deer. The neutralization titers varied between 1:5 and 1:125.

Among the free-living deer, we observed antibodies against BVDV in sera from seven (7.4%) of 94 deer from habitats with high cattle density (Schleswig-Holstein), four (9%) of 44 deer from habitats with intermediate cattle density (Bavaria) and three (7.1%) of 42 deer from habitats with low cattle density (Berlin). These antibody prevalences were not significantly different (P = 0.934).

Based on a paired comparison of antibody prevalence in roe deer (12 of 123 positive), fallow deer (one of 87), and red deer (three of 60), there was a significant difference between roe deer and fallow deer ($P = 0.009$), whereas the differences between roe deer and red deer ($P = 0.392$) and fallow deer and red deer ($P = 0.303$) were not significant; the significance level was adjusted according to Holm (1979). In roe deer, antibody positive sera were found in nine (14.7%) of 61 juveniles and in two (3.2%) of 62 adults; this difference was significant ($P = 0.030$). We found BVDV antibodies in 14 (7.7%) of 180 free-ranging animals, but only in four (2.3%) of 175 animals in wildlife parks or Berlin zoos; this difference was significant ($P = 0.027$). We divided the annual cycle into summer (April to September) and winter (October to March). In free-ranging cervids, we found antibodies in nine (14%) of 65 deer in summer but only in five (4.3%) of 115 deer in winter; this difference was significant ($P = 0.039$). The number of positive sera detected by means of Grub 313/83 strain was higher ($n = 15$) compared with NADL strain ($n = 9$). Significant differences in antibody prevalence by sex in all deer species were not found.

Based on our results, we believe that free-ranging deer can become infected with BVDV without having contact with cattle. Not all possible influencing factors were considered among the three habitats such as varying habitat types. Liebermann et al. (1989), Elazhary et al. (1981), and Weber et al. (1982) also assumed an independent infection process among wild ruminants with BVDV. In contrast, Romvary (1965), Neumann et al. (1980), and Kocan et al. (1986) assumed a causal relationship between the massive spread of BVDV in cattle and its occurrence in deer.

There were significantly more antibody-positive roe deer than fallow deer. Since the densities of roe deer and fallow deer in the study areas did not seem to differ considerably, based on the questionnaire to local foresters, we believe that density is of minor importance in transmission. Roe deer may be more susceptible to pestivirus strains circulating in the area than fallow deer, but this is not known. However, Stubbe (1981) reported that roe deer in general are very susceptible to diseases. There were significantly more seropositive juvenile than adult roe deer. The neutralization test was performed with a strain of BVD that does not exist in game animals and, therefore, is defined as a heterologous system. Perhaps the immune response in juveniles was more intense than in adults. Bovine viral diarrhea appears to be of minor importance in captive cervids compared with free-ranging deer, although the population density, and thus the possibility for transmission, is higher in enclosures. There may be a natural focus of infection for free-ranging cervids. Wild boar (*Sus scrofa*) and rabbit (*Oryctolagus cuniculus*) are potential carriers of BVD virus (Wizigmann, 1984). Antibody prevalence was significantly higher in summer than in winter and may be due to foraging behavior. Indigenous cervids forage much more frequently on meadows and pastures in summer than in winter (Kurt, 1991). Therefore, the risk for transmission of infection might be less in winter, when they are not grazing much from the ground.

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