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Bovine Virus Diarrhea Virus in Free-Living Deer from Denmark

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ABSTRACT: Free-living deer are suggested as a possible source of infection of cattle with bovine virus diarrhea (BVD) virus. To examine this hypothesis blood samples from 476 free-living deer were collected during two different periods and tested for BVD virus and antibody in Denmark. In 1995–96, 207 animals were tested. These included 149 roe deer (*Capreolus capreolus*), 29 fallow deer (*Dama dama*), 20 red deer (*Cervus elaphus*) and one sika deer (*Cervus sika*). For the remaining eight animals no species information was available. In 1998–99, 269 animals were tested including 212 roe deer and 57 red deer. The animals were selected from areas with a relatively high prevalence of cattle herds with a BVD persistent infection status in 1997 and 1998. All 207 samples from 1995–96 were found antibody-negative except two samples from red deer. Only 158 of the 207 samples were tested for virus and were all found negative. Of the 269 samples from 1998–99 all but one were antibody negative. The positive sample was from a red deer. All samples were virus-negative. It appears that BVD infection does not occur in roe deer in Denmark. The presence of antibody in a few red deer from various districts in Jutland probably results from cattle to deer transmission, rather than spread among deer. Hence, the possibility of free-living deer as a source of infection for cattle in Denmark seems to be remote.

Key words: Bovine virus diarrhea, dairy cattle, epizootiology, free-ranging deer, transmission.

Bovine virus diarrhea virus (BVDV) belongs to the genus *Pestivirus* of the family Flaviviridae. BVDV is generally seen in ruminants only and the main host is considered to be cattle. Only a few papers report the isolation of a BVD virus from other ruminants than cattle, namely red deer (*Cervus elaphus*) (Nettleton et al., 1980), fallow deer (*Dama dama*) (Neumann et al., 1980) and roe deer (*Capreolus capreolus*) (Romvary, 1965; Schellner, 1977). Only Frölich and Hoffmann (1995) have reported cytopathogenic BVDV in deer. Further characterization of these isolates referred them to BVD virus group 1, but subgrouping into subgroups 1a and 1b was not found feasible. The isolates were not considered as classical BVDV strains (Fischer et al., 1998). Antibodies to BVD virus have been found in sika deer (*Cervus nippon*) and white-tailed deer (*Odocoileus virginianus*) (Davidson and Crow, 1983), in roe deer, red deer and fallow deer (Dedek et al., 1988; Müller et al., 1997), mostly without finding of virus in the material investigated. The concentration of antibody has been reported in a few cases. In a naturally infected red deer, a virus neutralizing antibody (VNA) titer in serum of 40 was obtained while intramuscular inoculation of the same species with a BVDV isolate from cattle induced a VNA titre to 160 (McMartin et al., 1977). In Germany, neutralizing antibodies in red and roe deer were reported with BVD VNA titers from 2 to 8 (Dedek et al., 1988).

In Denmark, all cattle herds are under control for BVD (Bitsch and Roensholt, 1995) and by January 1999, 9% of dairy herds and 5% of beef herds were still registered to have a BVD persistent infection status (V. Bitsch, unpubl. data). In 1998, 204 Danish dairy herds were found infected after earlier having been found BVD-free. In some cases the cause of infection was well known, while in some other cases free-living deer were suggested to be the source of infection (V. Bitsch, unpubl. data). At the time of this investigation there was approximately 12,000 dairy herds in Denmark. The purpose of the present study was to investigate the prevalence of antibodies to BVD virus in the free-living deer in Denmark to determine

TABLE 1. Number of sampled deer of each species tested for bovine virus diarrhea virus in each forest district of Denmark.

Forest district	Longitude and latitude (center of district)	1995–96					1998–99	
		Roe deer	Red deer	Fallow deer	Sika deer	No info	Roe deer	Red deer
Frederiksborg (FR)	56°0′E, 12°10′N	69	0	28	1	0	—	—
Fussingø (FU)	56°28′E, 9°59′N	5	0	0	0	5	—	—
Klosterheden (KL)	56°29′E, 8°23′N	0	3	0	0	0	—	—
Aabenraa (ÅB)	54°55′E, 9°3′N	18	2	0	0	0	—	—
Haderslev (HA)	55°24′E, 9°21′N	44	0	0	0	0	76	0
Ulfborg (UL)	56°11′E, 8°23′N	0	15	0	0	0	17	20
Silkeborg (SI)[a]	56°8′E, 9°50′N	—	—	—	—	—	3	0
Buderupholm (BU)	56°50′E, 9°44′N	—	—	—	—	—	12	0
Falster (FS)[a]	55°21′E, 11°45′N	—	—	—	—	—	14	0
Feldborg (FE)	56°28′E, 9°0′N	—	—	—	—	—	30	5
Lindet (LI)	55°10′E, 8°56′N	—	—	—	—	—	21	1
Nordjylland (NJ)	57°23′E, 10°39′N	—	—	—	—	—	5	0
Odsherred (OD)	55°36′E, 11°32′N	—	—	—	—	—	31	0
Oxbøl (OX)	55°44′E, 8°23′N	—	—	—	—	—	0	31
Palsgaard (PA)[a]	55°57′E, 9°8′N	—	—	—	—	—	3	0
No information		13	0	1	0	3	—	—
Total		144	20	29	1	8	212	57

[a] SI, FS, and PA samples were collected by local hunters.

if deer are a potential treat to control of the disease in cattle.

The investigation was carried out in two time periods: November 1995 to January 1996 and November 1998 to January 1999. In the first period, blood samples were collected from six state forest districts in Zealand and Jutland. These districts were Frederiksborg (FR), Haderslev (HA), Klosterheden (KL), Aabenraa (ÅB), Fussingø (FU), and Ulfborg (UL) (Table 1). In 1998–99, samples were from eight state forest districts selected from areas with a relatively high number of newly infected herds. These districts were Nordjylland (NJ), Buderupholm (BU), Feldborg (FE), Oxboel (OX), Lindet (LI), Odsherred (OD), UL, and HA (Table 1). Furthermore, a few samples were collected from three areas, where local hunters showed interest in the study.

In 1995–96, 207 samples were collected including 149 from roe deer, 29 from fallow deer, 20 from red deer and one from a sika deer (*Cervus sika*). For the remaining eight samples no species information was received. In 1998–99, 269 samples were received consisting of 212 samples from roe deer and 57 samples from red deer.

The serum samples from 1995–96 were tested at the Danish Veterinary Institute for Virus Research (Lindholm, Denmark). After centrifugation and sterile filtration of the serum samples, they were inoculated onto secondary calf kidney cell culture. The harvests consisting of freeze/thaw disintegrated cells and media were tested 5 to 7 days after inoculation for presence of BVD virus using an antigen capture ELISA (Roensholt et al., 1996). Of the 207 samples, 57 were not tested for virus.

All samples from 1995–96 were tested for antibody against a type 1 BVDV strain using a blocking ELISA (Roensholt et al., 1996). Positive samples also tested against a type 1 BVDV strain using a virus neutralization test (Kamstrup et al., 1999). The blood samples from 1998–99 were tested at the Cattle Health Laboratory (Brørup, Denmark) by the ELISAs mentioned above, except that the samples were prepared a little different for the ELISA used for virus detection. This preparation

procedure was changed as follows: After removal of the plasma fraction from the centrifuged blood samples, 100 μl Triton-X-100 (from Merck, Darmstadt, Germany) was added to each sample and incubated at 20 C for 90 min. The rest of the ELISA procedure was done as previously. Antibody-positive samples were further tested in VNA tests at the Danish Veterinary Institute for Virus Research.

All samples tested for virus were found to be virus-negative. Of the 207 samples tested in 1995–96 for antibody, only two samples were positive. They showed VNA titers of 23 and 45, respectively. Both samples were from female red deer, one from the Klosterheden (KL) district and the other from the Ulfborg (UL) district. All samples but one from 1998–99 were antibody-negative. The positive sample showed a VNA titer of 16. The sample again was from a female red deer from the Feldborg (FE) district. All samples were found virus-negative.

We attempted to determine whether free-living deer could be contaminated from domesticated ruminants and be a source of BVD infection in cattle. Three red deer had antibodies to BVD virus, all with relatively low titers. Low titers of antibodies to BVD virus are reported previously in free-living deer (Dedek et al., 1988) and it was assumed that these low titers were caused by infection with traditional cattle strains. Higher titers would be expected if the causal BVD strain had been adapted or belonged to the actual deer species, though to be proven this might also demand a specific virus strain for the test in use (McMartin et al., 1977). Though the level of the VNA results might depend on the virus strain used, the antibody ELISA seems to be more crossreactive than the VNA and covers all BVDV strain of cattle so far (Roensholt et al., 1996). Thus, the results obtained gave no reasons to suspect the occurrence of a BVD infection, which was adapted for and circulated among free-living roe and fallow deer in Denmark. The finding of three of 57 (5%) antibody-positive red deer shows that this species is susceptible to the infection. However, if red deer were a source of infection for cattle, a higher number of BVD antibody-positive deer would be expected. The low number of antibody-positive red deer and the fact that the titres were low as compared to these in cattle suggest that infection in deer is possible, but that transmission among deer or from deer to cattle is highly unlikely.

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