Pestivirus infections in cervids from the Czech Republic

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ABSTRACT: 372 sera of cervids from the Czech Republic were examined for antibodies to the bovine viral diarrhea virus (BVDV) and border disease virus (BDV) by competitive-inhibition enzyme-linked immunosorbent assay (ELISA), and for the presence of the BVDV by AgELISA. Antibodies to BVDV/BDV were found in 0.6% (two positive/305 tested) red deer (*Cervus elaphus*). BVDV/BDV antibodies were not found in four sika deer (*Cervus nippon*) and 63 fallow deer (*Dama dama*). All serum samples were BVDV antigen negative. Our results confirmed that red deer in the Czech Republic are only rarely infected with Pestiviruses. This was the first survey of pestiviruses in farmed and wild cervids in the Czech Republic.

Keywords: bovine viral diarrhea virus; red deer; fallow deer; wildlife; prevalence; ELISA

Bovine viral diarrhea (BVD) is a contagious disease caused by a *Pestivirus* of the Flaviviridae family. Pestiviruses are prevalent worldwide and are important pathogens of domestic cattle, sheep and pigs. Pestivirus infections include BVD and mucosal disease in cattle, border disease (BD) in sheep and classical swine fever in pigs. Moreover, pestivirus was isolated from a giraffe and various species of wild ruminants with a syndrome similar to mucosal disease. However, the subclinical form of the diseases usually develops (Vilcek and Nettleton, 2006; Thiry, 2007). A novel pestivirus, causing systemic lethal disease in a Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*), was isolated and classified as border disease virus (BDV) type 4 (Frolich et al., 2005).

Pestiviruses have been detected in several cervids, including red deer (Nettleton et al., 1980), roe-deer (Frolich and Hofman, 1995), reindeer (Becher et al., 1999), caribou (Elazahary et al., 1981), and moose (Thorsen and Henderson, 1971). Surveys on bovine viral diarrhea virus (BVDV) antibodies have been conducted in various deer species. Antibodies to BVDV were found in red deer, roe-deer, fallow deer, reindeer and moose in various European countries (Frolich, 1995; Frolich and Flach, 1998; Cuteri et al., 1999; Lillehaug et al., 2003).

In the Czech Republic, BVDV is commonly prevalent in the cattle population, however, little is known about the prevalence of pestiviruses in wildlife. The aim of this study was to survey BVDV/BDV prevalence in both farmed and wild cervids in the Czech Republic.

MATERIAL AND METHODS

Blood sampling

During the period 1999–2006, sera from 372 cervids from eight of the Czech Republic's 14 regions (Prague, Stredocesky, Plzensky, Jihocesky, Ustecky, Pardubicky, Moravskoslezsky and the Vysocina Region) were collected. Wild cervids came from game-preserves, farms or were free-ranging.

Blood samples were taken from 305 red deer (*Cervus elaphus*) coming from three farms, three game-preserves and in one case from free-ranging animals, four sika deer (*Cervus nippon*) coming from one game-preserve and 63 fallow deer coming from five game-preserves. The blood samples were obtained from animals before transportation. The examined animals were clinically healthy adults, but detailed sex and age data was not available.

Enzyme-linked immunosorbent assay (ELISA)

Blood was centrifuged and sera were stored at –20°C until assayed for antibodies to BVDV/BDV and BVDV antigen. A commercial competitive-inhibition enzyme-linked immunosorbent assay (ELISA) Pourquier ELISA BVD/MD/BD P80 Antibody Screening (Institute Pourquier, France) was used for the detection of BVDV and BDV antibodies in wild cervids according to the manufacturer's instructions. The sera were positive, if 40% or less inhibition was found. All ELISA positive samples were retested using a virus neutralization test with Oregon Strain (BVDV 1 type). BVDV antigen was detected in sera by an AgELISA using a commercially available kit ELISA BVD/MD Antigen Mix (Institute Pourquier) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

The results of the survey of BVDV/BDV antibodies in cervids in the Czech Republic are presented in Table 1. In our study, we isolated specific BVDV/BDV antibodies in only two of 305 red deer tested. Inhibition in positive animals was 13.7% and 16.2%. The remaining cervid samples were found to be negative to antibodies and all the tested serum samples were negative to BVDV antigen.

Similar results were reported in Austria by Krametter et al. (2004). BVDV antibodies were found in 0.7% (one positive/145 tested) of wild ruminants. Only one of 59 tested red deer had antibodies to BVDV-1 and BDV (Krametter et al., 2004). In Norway, antibodies to BVDV were found in 1.1% red deer, 2.0% moose, 4.2% reindeer and 12.3% roe deer (Lilehaug et al., 2003). Recently, Frolich et al. (2006) examined 164 red deer, roe deer and fallow deer but they did not prove to have any specific antibodies to BVDV. We can state that the BVD seroprevalence in red deer in our present study is in agreement with the values found in other European countries. In contrast to other reports (Cuteri et al., 1999) we did not detect any seropositive fallow deer.

Both positive red deer were from one farm of 50 heads. Approximately 25 heads of meat cattle breed were also kept on the farm, but on a different pasture. The owner prevents the direct or indirect contact of the deer with the cattle. The BVDV status in cattle was unknown. The cattle sera will be tested for antibodies against BVDV in the near future and the seroprevalence will be established.

Antibodies against BVDV/BDV were analysed by ELISA screening. The commercial ELISA used in this study has been validated for bovine and ovine sera. This test can theoretically be used with any other species but validation data is not yet available. Both samples strongly positive by ELISA were negative by the virus neutralisation test with BVDV-1. The different results can be explained by the low sensitivity of the virus neutralisation test compared to ELISA and the different serotype selectivity of both tests. Neutralisation tests for BVDV-2 antibodies and antibodies against other pestiviruses were not provided. The question of whether pestivirus may be responsible for seroconversion in both deer still remains to be answered.

From our results we can conclude that the prevalence rate of pestiviruses in cervids in the Czech Republic is very low. Therefore we suppose that any danger of the introduction of this disease in cattle herds kept on pastures is almost negligible. Further investigation and research has to be undertaken to prove this hypothesis. The detection of specific antibodies and virus antigens from serum samples

Table 1. The prevalence of antibodies to BVDV in cervids in the Czech Republic

Species	Samples (*n*)	Breeds (*n*)	Positive (*n*)	Prevalence (%)
Red deer (*Cervus elaphus*)	305	7[a]	2	0.6
Sika deer(*Cervus nippon*)	4	1[b]	0	0
Fallow deer (*Dama dama*)	63	5[c]	0	0
Total	372	13	2	0.5

[a]samples originated from three farms, three game preserves, and additionally, one sample was from free ranging deer

[b]samples originated from one game preserve

[c]samples originated from five game preserves

by ELISA is economically feasible and easy to carry out. Both methods enable monitoring of BVDV/BDV infection; however, testing to directly detect the virus, especially RNA from tissue samples (i.e., spleen), are recommended.

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