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*Original article*

# Detection of seroconversion to bovine herpesvirus 1 related alphaherpesvirus and bovine viral diarrhoea virus in Polish free-living deer

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## Abstract

There are several infectious agents of domestic cattle that can also be present in free-living ruminant populations. These include bovine herpesvirus 1 (BoHV-1) and bovine viral diarrhoea virus (BVDV) which are the causative agents of infectious bovine rhinotracheitis and bovine viral diarrhoea, respectively. The study was conducted on serum samples from 59 red deer, 24 roe deer, and 3 fallow deer (86 in total), originating from two geographically separate areas of Poland. The samples were tested with commercially available ELISA tests for BoHV-1 and BVDV. The overall seroprevalence was 5.8% and 3.5%, respectively. All positive samples originated exclusively from red deer. Because of BoHV-1 ELISA cross reactivity with cervid herpesvirus 1 and 2 (CvHV-1 and -2) the nature of alphaherpesviruses infecting the sampled animals could not be assessed.

**Key words:** BVDV, BoHV-1, alphaherpesvirus, deer, ELISA, antibodies

## Introduction

Many infectious agents of domestic animals are shared with free living animals. Transmission of these agents has been documented to occur from wildlife to livestock, and vice versa (Gortazar et al. 2007). In North America, antibodies to important viral pathogens of farm ruminants, such as bovine viral diarrhoea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), epizootic

haemorrhagic disease virus (EHDV), parainfluenza virus type 3, bluetongue virus (BTV), and bovine respiratory syncytial virus (BRSV) have been detected in free-deer species (Conner et al. 2008).

BVDV is a member of the genus Pestivirus, family Flaviviridae, and can infect ruminants, as well as pigs (Lanyon et al. 2014). The infection is predominantly transmitted by direct contact. BoHV-1 is a member of the genus Varicellovirus, subfamily Alphaherpesviri-

Table 1. Summary results of serum sample tested with ELISA for BoHV-1 and BVDV antibodies.

		Number of animals	BoHV-1 positive	BVDV positive
site A	red deer	29	0	3
	roe deer	3	0	0
site B	red deer	30	5	0
	roe deer	21	0	0
	fallow deer	3	0	0

nae, family Herpesviridae and can infect cattle as well as deer (Muylkens et al. 2007). Similarly to BVDV direct contact is the most important method of transmission of BoHV-1. The negative impact of BVDV and BoHV-1 on cattle production has resulted in control and elimination program implementation in many countries (Presi et al. 2011). Efficiency of these programs differs between countries (Heffernan et al. 2009). Detection of BoHV-1 and BVDV infections in free-living ruminants might indicate their role as a natural reservoir possibly compromising disease eradication efforts.

The seroprevalence of BoHV-1 and BVDV in free-living deer populations was a subject of several studies conducted in Europe. They showed a high diversity of seroprevalence in different deer species, from 0 to 14% for BoHV-1 and from 0% to 6.5% for BVDV (Lillehaug et al. 2003, Frolich et al. 2006). In a very recent study in Ireland BVDV and BoHV-1 were detected in 1.5% and 1.8% of the sampled deer, respectively (Graham et al. 2017).

In Poland seroreactivity to bacterial and viral pathogens in free-living ruminants was intensively studied in the European bison population (Salwa et al. 2007). In a recent study on BoHV-1 related alphaherpesvirus infections Rola et al. (2017) found 17.9% of free-living cervids positive in ELISA. In Poland the most common deer species are red deer, roe deer and fallow deer. The density of deer species has significantly increased in recent years and so has the risk of infection transmission between free living and domestic ruminants. Considering the above, the aim of this study was to analyse the seroprevalence of BoHV-1 related alphaherpesvirus and BVDV in free-living deer species in Poland.

## Materials and Methods

The study was conducted on 86 serum samples obtained from free-living wild cervids. The samples were collected during hunting at two separated sites, in the 2009/2010 season. Thirty-two samples were collected from site A located in the North-East of Poland (lati-

tude 53°46' North, longitude 21°26' East) and 54 samples were collected from site B located in Central Poland (latitude 51°35' North, longitude 20°16' East). The distance between these two sites is approximately 300 km in a north-south direction. The sampled group consisted of 29 red deer and 3 roe deer from site A, and 30 red deer, 21 roe deer, and 3 fallow deer from site B.

Blood samples were collected immediately after the animals were shot, allowed to clot, and then transported to the laboratory, chilled. After delivery to the laboratory, serum was separated by centrifugation (2400 × g for 8 min.), transferred to Eppendorf tubes and stored at -20°C until assayed.

The detection of antibodies against BoHV-1 and related alphaherpesviruses was performed using HerdChek IBRgB ELISA (IDEXX, Switzerland), and the detection of specific antibodies against BVDV was performed using HerdChek BVDV Ab ELISA (IDEXX, Switzerland). Both tests were performed according to the manufacturer's protocols.

## Results

Five out of 86 (5.8%) samples reacted positive in BoHV-1 ELISA and three samples out of 86 (3.5%) reacted positive in BVDV ELISA (Table 1). All positive samples were obtained only from red deer, while samples from roe deer and fallow deer reacted negative. Thus, 8.5% and 5.1% of red deer samples were positive for BoHV-1 and BVDV antibodies, respectively. However, all BoHV-1 positive animals originated from site B and all BVDV positive animals originated from site A.

## Discussion

In the present study on 86 serum samples, collected in two separate regions of Poland, and obtained from three main deer species, we found 5.8% of the animals reacting positive in BoHV-1 ELISA, and 3.5% of the animals reacting positive in BVDV ELISA. All positive

samples originated from red deer, but all BVDV positive animals originated from site A, and all BoHV-1 positive animals originated from site B.

In Austria Krametter et al. (2004) found one BVDV positive red deer sample out of 147 animals tested (0.7%). In Denmark Nielsen et al. (2000) found BVDV antibodies in two out of 207 animals tested in the 1995/1996 season, and in one out of 269 animals tested in the 1998/1999 season. This latter study included roe deer, red deer, and fallow deer but only red deer individuals were found positive. A relatively high seroprevalence (4.5%) of BVDV was detected in central Italy (Cuteri et al. 1999) and the antibodies were found in fallow deer. The difference in distribution of seroreagents to BoHV-1 and BVDV in various deer species, was also observed in other studies (Frolich et al. 1995, 2002, 2006). In the first study (Frolich, 1995) 28.3% of the tested free living and captive red deer were positive for BVDV antibodies, while the remaining deer species tested negative. In contrast, in the latter study, performed on samples obtained from free living cervids from national parks (Frolich et al. 2006), the authors found no animals positive to BVDV, and BoHV-1 antibodies were detected in 28% of red deer, 16% of roe deer and in 2% of fallow deer samples. In the study performed in Norway nearly 4000 samples collected between 1993 and 2000 were tested for BoHV-1 and BVDV seroconversion (Lillehaug et al. 2003). BoHV-1 antibodies were detected in 3% of samples from roe deer, and in 0.5% of samples from red deer. Antibodies against BVDV were detected in 12.3% of roe deer and 1.1% of red deer. There were significant differences in geographical distribution of the seroprevalence. For example, BoHV-1 antibodies were detected in from 1% up to 25% of roe deer samples from different regions of Norway. Similarly, BVDV seroconversion ranged from 7% to 25% of roe deer. In Poland seroconversion to BoHV-1 related alphaherpesvirus was analyzed in free-living and captive cervids and 17.9% and 38.5% of samples were found to react positive in BoHV-1 ELISA, respectively (Rola et al. 2017). The differences in results obtained in different countries are difficult to explain but they probably originate from different sampling protocols and differences in the ecology of local populations.

An extensive serological cross reactivity between ruminant alphaherpesviruses, including cervid herpesvirus 1 and 2 (CvHV-1 and -2) is a limitation to studies employing gB ELISA for BoHV-1 (Lillehaug et al. 2003, Thiry et al. 2006, Rola et al. 2017). Also, some cross reactivity between ruminant alphaherpesviruses can be observed in virus neutralization tests (Rola et al. 2017). As a result, it is difficult to ultimately define the identity of the alphaherpesvirus species inducing sero-

conversion detected with the BoHV-1 ELISA. Infection with CvHV-1 is considered to be widespread in Europe (Thiry et al. 2007), particularly in red deer. In a recent study Rola et al. (2017) performed virus neutralization tests on 177 serum samples from free-living and captive cervids, that reacted positive in BoHV-1 ELISA and found all of them to react with significantly higher titers against CvHV-1 than BoHV-1. These results suggested that the former virus is dominant in wild cervids and that the role of deer as a natural reservoir of BoHV-1 is likely marginal. Potential routes for herpesvirus transmission within and between species of wild ruminants and between wild ruminants and cattle are not fully understood, but it can be assumed that as between farm animals direct contact would be required. This applies also to BVDV. Husbandry practices in cattle production and the high density of animals facilitate virus transmission and eventually environment contamination. Thus, it can be speculated that farm animals are more likely to act as a source of BoHV-1 and BVDV for wildlife, than vice-versa.

Our study provides evidence on BoHV-1 related, alphaherpesvirus and BVDV infections in free-living deer species in Poland. Although the number of tested animals was low, red deer was found to be the only species harboring viruses potentially significant for cattle. Our results indicate that geographically broader and systematic studies are needed to fully assess the ecology of BVDV and alphaherpesviruses in free living ruminants, as well as their precise genetic and antigenic characterization.

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