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The occurrence of alpha-herpesvirus and pestivirus infections in European bison (*Bison bonasus*) in the Bialowieza Primeval Forest

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Abstract: Serological survey on the occurrence of alpha-herpesvirus and pestivirus infections in the population of European bison in the Bialowieza Primeval Forest was conducted. Sera from 40 randomly selected individuals were tested using two commercial IDEXX ELISA tests. The study design allowed excluding or confirming the infection with the probability of 95% and at the expected prevalence of 30%. No antibodies either to alpha-herpesviruses (BHV-1, BHV-5, BuHV-1, CpHV-1, CvHV-1, CvHV-2, ElkHV-1) and pestiviruses (BVDV-1, BVDV-2, BDV) were detected. The results of the study imply that Polish population of European bison is unlikely to have a contact with any of the viruses.

Key words: herpesvirus, pestivirus, European bison

Introduction

The alpha-herpesvirus subfamily gathers many similar viruses, called from their main mammal hosts. Apart from bovine herpesvirus type 1 (BHV-1), currently six other herpesviruses are deemed capable of infecting ruminants. These are: bovine herpesvirus 5 (BHV-5) responsible for severe meningoencephalitis in calves, bubaline herpesvirus 1 (BuHV-1) inducing a subclinical genital infection in water buffalo (*Bubalus bubalis*), caprine herpesvirus 1 (CpHV-1) causing systemic disease in young kids and abortion in adult goats, cervid herpesvirus 1 (CvHV-1) responsible for severe ocular syndrome in red deer (*Cervus elaphus*), cervid herpesvirus 2 (CvHV-2) and elk herpesvirus 1 (ElkHV-1), which induce a subclinical genital infection respectively in reindeer (*Rangifer tarandus*) and elk (*Cervus canadensis*) (Thiry *et al.* 2006).

Bovine viral diarrhea in wild ruminants may be caused by one three ruminant pestiviruses – bovine viral diarrhea virus type 1 (BVDV-1), bovine viral diarrhea virus type 2 (BVDV-2) and border disease virus (BDV) (Martin *et al.* 2011). Pestiviruses are widespread in populations of domestic and wild ruminants and they have already been isolated from European bison (Deregt

et al. 2005). Both herpesvirus and pestivirus are able to survive in the organisms of their hosts. The former causes latent infection and remains dormant in the nervous cells, whereas the latter produces persistent infection, being recognized as the host's own antigen if the infection is established during prenatal life. The infections pose a substantial problem since they may be eradicated only by culling infected animals (Moening *et al.* 2005; Rypuła *et al.* 2010; Thiry *et al.* 2006).

Given high prevalence of herpesvirus and pestivirus infections in domestic ruminants, the study was performed to assess the epidemiological situation in the population of European bison in the Bialowieza Primeval Forest, the main refuge of this animal in Poland.

Materials and methods

In total 40 European bison, 26 females and 14 males, were enrolled for the study. They were all free-ranging adults, between 6 months and 20 years of age. The animals were randomly selected from the entire population of European bison in the Bialowieza Primeval Forest, which counted 451 individuals – 254 females and 197 males. The study sample was calculated for the expected seroprevalence of 30% and the level of confidence of 95%, according to the following formula:

$$n = \left[1-(1-p_1)^{t/d}\right]\times\left(N-\frac{d}{2}\right) + 2$$

where: n – required sample size; p_1 – expected seroprevalence; N – population size; d – number of infected animals in a population.

Blood samples were collected from the animals and sent to the diagnostic laboratory „Epi-Vet" at the Department of Epizootiology with the Clinic for Birds and Exotic Animals, Wrocław Faculty of Veterinary Medicine. Then, serum was obtained by centrifuging the blood samples and tested using two immunoenzymatic assays: HerdChek BHV-1 gB (IDEXX Scandinavia AB, Sweden), searching for antibodies to the glycoprotein B and HerdChek BVDV Ab (IDEXX Scandinavia AB, Sweden) directed against antibodies to the protein p80. The ELISA tests were performed according to the manufacturers' manuals.

Results

No antibodies against either BHV-1 or BVDV were revealed in the serum of examined animals. Detailed results are provided in table 1.

Discussion

Glycoprotein B is a conservative particle of the alpha-herpesviruses common for all the representatives of the subfamily. Therefore, it can be used for initial verification of exposure to the alpha-herpesviruses in ruminant populations.

Table 1. Results of serological examination of the European bison (*Bison bonasus*) for alpha--herpesvirus and pestivirus infections

Pathogen	Result	European bisons (n=40)	
		male	female
BHV-1	positive	0	0
	negative	14	26
BHV-5	positive	0	0
	negative	14	26
BuHV-1	positive	0	0
	negative	14	26
CaHV-1	positive	0	0
	negative	14	26
CvHV-1	positive	0	0
	negative	14	26
CvHV-2	positive	0	0
	negative	14	26
ElkHV-1	positive	0	0
	negative	14	26
BVDV	positive	0	0
	negative	14	26

Negative result in anti-gB BHV-1 test means that no antibodies to BHV-5, BuHV-1, CaHV-1, CvHV-1, CvHV-2 and ElkHV-1 were found as well.

First reports on the occurrence of BHV-1 in wild ruminants in Europe are sourced from Finland, where 23% of Finnish reindeer were seropositive (Ek-Kommonen *et al.* 1982). In subsequent years the infections were reported from other European countries such as Belgium, Czech Republic, France, Germany, and Italy (Pospisil *et al.* 1996; Muller *et al.* 1997; Kalman and Egued 2005; Thiry *et al.* 2006a; Thiry *et al.* 2007). However, the problem of cross-species herpesvirus infections among wild ruminants dates back to sixties and seventies of the XX century, when BHV-1 infections were reported from Africa in many species of the subfamily *Bovinae* such as domestic cattle (*Bos taurus*), water buffalo (*Bubalus bubalis*) African buffalo (*Syncerus caffer*) oraz Nyala (*Tragelaphus angasii)*, greater kudu (*Tragelaphus strepsiceros)*, buschbuck (*Tragelaphus scriptus*) and eland (*Taurotragus oryx*). The highest seroprevalence of 30% was found in common eland and African buffalo, lower, of 12–14%, in sable antelope (*Hyppotragus niger*), impala and kudu (*Tragelaphus strepsiceros*) and the lowest in bushbuck (*Tragelaphus scriptus*), nyala (*Tragelaphus angasii*) and tsessebe (*Damaliscus lunatus*) (Barnard 1977; Anderson and Rowe, 1998). Moreover, in the North America around 38% and 44% of free-ranging

and ranch-raised American bisons (*Bison bison*), respectively, were seropositive (Taylor *et al.* 1977; Sausker and Dyer 2002). The BHV-1 seroprevalence in white-tailed deer (*Odocoileus virginianus*) ranged between 15% in Minnesota and 57% in Quebec (Ingebrigtsen *et al.* 1986; Sadi *et al.* 1991).

In Poland serological studies on the occurrence of alpha-herpesviruses in the population of European bison were carried out at the beginning of the XXI century (Borchers *et al.* 2002). None of examined animals (n=234), had antibodies against BHV-1, BHV-4, CpHV-1 and CvHV-1, whereas only two bisons had a antybody against BHV-2. Our present study confirms that the epidemiological situation in the population is constant. It is very hard to decide if there is any risk of herpesvirus infections for the population of European bison in Poland as serological surveys for herpesvirus infections including ruminants other than cattle are lacking. However, the study conducted in 2007 on the population of Polish breeding goats did not provide any serological evidence of the contact with herpesviruses in this ruminant species (Czopowicz *et al.* 2010).

A search for pestivirus antibodies and virus isolates in ruminants other than cattle has been conducted intensively for the last 20 years. In Germany antibodies to BVDV were found in free-ranging and captive ruminants, and the seroprevalence was significantly higher in the former than in the latter group (Frolich 1995). Serological study carried out in Norwegian cervids revealed prevalence of 4.9% (Lillehaug *et al.* 2003). Around 13% of serum samples collected between 1973 and 1994, in the UK from free-living and captive European bison, scimitar--horned oryx, Pere David's deer were positive for antibodies to BVDV (Frolich and Flach 1998). In the French and Spanish Central Pyrenees prevalence was 16% but in Andorra and Benasque as much as 27% of animals were seropositive (Arnal *et al.* 2004). On the other hand, in Denmark only three red deer samples out of 476 tested over the period 1995–1999 were diagnosed as positive for antibodies to BVDV (Nielsen *et al.* 2000). As much as 31% of free-ranging American bison, whereas only 0.6% of European bison were seropositive to BVDV (Taylor *et al.* 1997; Borchers *et al.* 2002). The cross-species infections with BVDV-1 were serologically confirmed for breeding goats in Poland (Czopowicz *et al.* 2011).

The ELISA test applied in the study allows detecting antibodies to all three species of ruminant pestiviruses as p80 is a common pestiviral protein. Negative test result means that no antibodies to BVDV-1, BVDV-2 and BDV were found as well.

The share of ecological niches with other ruminant species is the main factor that can contribute to the transmission of pestiviruses and alpha--herpesviruses between cattle and other ruminant species. Direct or indirect contact between potential wildlife hosts and livestock is likely to occur on common pastures, water holes and feeders. Moreover, the current development of breeding of ruminants sourced from wild fauna also plays its role. Once a wild ruminant becomes infected, the viruses will persist in a population until latent carrier have died or been eliminated from the population. Recently,

such transmission in red deer has been assessed under experimental conditions based on the basic reproduction ratio (R0) (Mollema *et al.* 2005). This parameter describes infection dynamics in a population and is defined as the average number of secondary cases generated by one primary case in a fully susceptible population of defined density (Muylkens *et al.* 2007). In a dairy cattle herd, R0 was estimated to be at least 7. For red deer, the "worst case scenario" was discussed and the R0 was estimated at 1.2. The authors concluded that most likely red deer will be a satellite group for BHV-1 because the virus will not survive longer than a few decades (several times the average deer lifespan) in red deer populations (Hage *et al.* 1996; Mollema *et al.* 2005).

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Występowanie infekcji alpha-herpeswirusów i pestiwirusów u żubrów (Bison bonasus) w Puszczy Białowieskiej

Streszczenie: Przeprowadzono badania serologiczne występowania infekcji alpha-herpeswirusami i pestiwirusami u żubrów z Puszczy Białowieskiej. Surowice 40 losowo wybranych osobników testowano przy użyciu dwóch komercyjnych testów IDEXX ELISA. Układ doświadczenia pozwalał na potwierdzenie lub wykluczenie infekcji z prawdopodobieństwem 95% przy przewidywanym zakażeniu na poziomie 30%. Nie stwierdzono przeciwciał przeciwko alpha--herpeswirusom (BHV-1, BHV-5, BuHV-1, CpHV-1, CvHV-1, CvHV-2, ElkHV-1) ani pestiwirusom (BVDV-1, BVDV-2, BDV). Te wyniki badań sugerują, że polska populacja żubra najprawdopodobniej nie miała kontaktu z tymi wirusami.