The prevalence of specific antibody to selected viral and bacterial infections in wild ruminants in Poland

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Abstract

Serological and virological survey on the occurrence of α-herpesvirus (BHV-1, BHV-5, BuHV-1, CpHV-1, CvHV-1, CvHV-2, ElkHV-1), pestivirus (BVDV-1, BVDV-2) and Coxiella burnetii infections in the population of wild ruminants in Poland was conducted. Sera from 40 randomly selected European bison from the Bialowieza Primeval Forest and 32 representatives of other wild ruminant species were used in the study. The sera were tested using commercial ELISA tests for antibodies against α-herpesviruses, pestiviruses and C. burnetii and Real Time PCR for pestivirus antigen. Only antibodies to pestiviruses were detected in the sera of two chital deer (Axis axis). No antibodies to α-herpesviruses and C. burnetii were found as well as the results of Real Time PCR were all negative. The results of the study imply that contact of Polish wild ruminants with these infections is very limited.

Key words: *prevalence, antibody, wild ruminants, of α-herpesvirus, pestivirus, Coxiella burnetii, serology, PCR.*

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Introduction

There are a few classical infectious diseases such as bovine leukemia, brucellosis and tuberculosis widely recognized as important threat to wild ruminants' health. However, last decade has shown that other pathogens, considered very dangerous for livestock may play important role in wildlife as well. These are herpesviruses belonging to α-herpesvirus subfamily – bovine herpesvirus type 1 (BHV-1), bovine herpesvirus type 5 (BHV-5), bubaline herpesvirus type 1 (BuHV-1), caprine herpesvirus type 1 (CpHV-1), cervid herpesvirus type 1 (CvHV-1), cervid herpesvirus type 2 (CvHV-2) and Elk herpesvirus type 1 (ElkHV-1) as well as bovine viral diarrhea virus type 1 and 2 (BVDV-1 and BVDV-2) [1, 2]. Moreover, Q fever, dangerous zoonosis caused by *Coxiella burnetii*, seems to be of growing importance, especially in Med-Vet-Net Association reports. Numerous foci of the disease, which have emerged in recent time in many European countries, indicate that the reservoir of the disease on the continent has to be wide and is certainly only partially recognized. Thus, endemic occurrence of Q fever in Europe has been postulated [3, 4].

Given the high prevalence of herpesvirus, pestivirus and *C. burnetii* infections in domestic ruminants in Europe, the study was performed to assess the epidemiological situation in the population of wild animals in Poland.

Material and methods

Seventy-two wild animals from Poland were sampled for the purpose of the study. Forty of them were European

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Table 1. Results of serological examination of the wild ruminants for α-herpesvirus, pestivirus and Q fever infections

Infectious agents	Antibodies to herpesviruses		Antibodies to pestiviruses		Antibodies to *Coxiella burnetii*	
Wild ruminant species	+ve	–ve	+ve	–ve	+ve	–ve
European bison (n = 40)	0	40	0	40	0	40
Roe-deer (n = 20)	0	20	0	20	0	20
Reindeer (n = 2)	0	2	0	2	0	2
Chital deer (n = 2)	0	2	2	0	0	2
Nilgau (n = 1)	0	1	0	1	0	1
Sitatunga (n = 1)	0	1	0	1	0	1
Common wildebeest (n = 1)	0	1	0	1	0	1
Aoudad (n = 5)	0	1	0	1	0	1

bison, 26 females and 14 males. 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:

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

where:

n – required sample size

p_1 – probability of detection at least one seropositive animal in a population

N – population size

d – number of seropositive animals in a population

The remaining 32 were roe-deer (*Capreolus capreolus*) (n = 20), reindeer (*Rangifer tarandus*) (n = 2), chital deer (*Axis axis*) (n = 2), nilgau (*Boselaphus tragocamelus*) (n = 1), sitatunga (*Tragelaphus spekii*) (n = 1), common wildebeest (*Connochaetes taurinus*) (n = 1) and aoudad (*Ammotragus lervia*) (n = 5).

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 three immunoenzymatic assays: HerdChek BHV-1 gB (IDEXX Scandinavia AB, Sweden), searching for antibodies to the glycoprotein B, HerdChek BVDV Ab (IDEXX Scandinavia AB, Sweden) directed against antibodies to the protein p80 and Q Fever Ab Test (IDEXX Europe B.V., Netherlands). The ELISA tests were performed according to the manufacturers' manuals.

Moreover, all serum samples were tested using real time PCR for genetic material of BVDV. Viral RNA isolation was performed using QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions. Reverse-transcription and real-time PCR was done on iQ5 system (BioRad) using ADIVENT BVD Real time kit (Biomedica, Austria).

Results

No antibodies against any of the α-herpesviruses or *C. burnetii* were revealed, whereas antibodies to BVDV were detected exclusively in two chital deer (*Axis axis*). Hence, seroprevalence of BVDV infection in wild ruminants was 2.8%. All results of serological examination for α-herpesvirus, pestivirus and Q fever infections show in Table 1. In Table 2 are results of examination of the wild ruminants for pestivirus antigen. No genetic material of BVDV in any serum sample was found.

Discussion

Glycoprotein B is a conservative particle of the α-herpesviruses common for all the representatives of the sub-

Table 2. Results of examination of the wild ruminants for pestivirus antigen

Infectious agents	Antigen of pestiviruses	
Wild ruminant species	+ve	–ve
European bison (n = 40)	0	40
Roe-deer (n = 20)	0	20
Reindeer (n = 2)	0	2
Chital deer (n = 2)	0	2
Nilgau (n = 1)	0	1
Sitatunga (n = 1)	0	1
Common wildebeest (n = 1)	0	1
Aoudad (n = 5)	0	1

family. Therefore, it can be used for initial verification of exposure to the α-herpesviruses in ruminant populations. 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 [5]. In subsequent years the infections were reported from other European countries such as Belgium, Czech Republic, France, Germany, and Italy [6-10]. 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 (*Hippotragus niger*), impala and kudu (*Tragelaphus strepsiceros*) and the lowest in bushbuck (*Tragelaphus scriptus*), nyala (*Tragelaphus angasii*) and tsessebe (*Damaliscus lunatus*) [11,12]. Moreover, in the North America around 38% and 44% of free-ranging and ranch-raised American bisons (*Bison bison*), respectively, were seropositive [13,14]. The BHV-1 seroprevalence in white-tailed deer (*Odocoileus virginianus*) ranged between 15% in Minnesota and 57% in Quebec [15, 16].

In Poland serological studies on the occurrence of α-herpesviruses in the population of European bison were carried out at the beginning of the XXI century [17]. None of examined animals (n = 234), had antibodies against BHV-1, BHV-4, CpHV-1 and CvHV-1, whereas only two bison had a antibody 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 [18].

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 [19]. 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 [21]. In the French and Spanish Central Pyrenees prevalence was 16% but in Andorra and Benasque as much as 27% of animals were seropositive [22]. 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 [23]. As much as 31% of free-ranging American bison, whereas only 0.6% of European bison were seropositive to BVDV [17,24]. The cross-species infections with BVDV-1 were serologically confirmed for breeding goats in Poland [25]. 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.

BVD control programs are being conducted in most European countries. Several countries have programs based solely on elimination of persistently infected animals (PI) and restrictive supervision over international and national turnover of animals [26-28]. However, no such methods can be applied to monitor status of wild ruminants in practice, while these animals may serve as a source of the virus for livestock. Particularly alarming is the emergence of new pestivirus genotypes, isolated from wild ruminants. Following BVDV infection these animals only seroconvert, remaining clinically healthy [29-32].

Q fever is a commonly recognized zoonosis. Its diagnosis is highly problematic due to subclinical course of infection in animals. However infected ruminants shed bacteria with milk, urine and feces. Particularly dangerous are placentas and fetal fluids, which contain pathogen in very high concentration, reaching 10^{12} cells per 1 g of the placenta [4, 33, 34]. According to the regulation of the Minister of Agriculture dated 24 June 2010 (Journal of Law 9 July 2010) Q fever is now subject to the mandatory monitoring in Poland. For the purpose of monitoring blood samples from cattle or sheep and goats are collected yearly, so that at least one seropositive animal could be found with 95% probability, if seroprevalence in population of a district is 25% [35]. Serological survey conducted in 2007 in breeding goat population in Poland did not reveal any seropositive goat [36].

The share of ecological niches with other ruminant species is the main factor that can contribute to the transmission of bacteria and viruses 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.

Evaluation of prevalence of the studied pathogens is an important part of disease control programs in livestock populations and, in the case of zoonozes, useful indicator of human risk linked to the contact with wild animals.

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