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# Serological survey of viral respiratory infections in European bison in Poland

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**Abstract:** European bison *(Bison bonasus)* were tested for the presence of antibodies to viral pathogens known to cause respiratory disorders in cattle, involved also in an etiological complex Bovine Respiratory Disease (BRD). The aim of this study was to evaluate the potential health threats for European bison population in Poland, as well as to assess the association between the viral infections and: animal age, gender, population type (free-living, captive), geographical origin, health status (fallen, culled or immobilized) and year of sampling. A total of 145 European bison from eight populations: Białowieża Primeval Forest (87 animals), Smardzewice (17), Bieszczady (12), Pszczyna (11), Borecka Forest (8), Gołuchów (5), Warsaw ZOO (4) and Strzelinko (1) were tested using commercial bovine ELISAs. Serum, plasma or *post-mortem* body fluids were collected from chemically immobilized for diagnostic purposes, post mortally examined eliminated or fallen European bison. Out of 145 and 144 animals only one (0.7%; 95%CI 0.1–3.8%) and two (1.4%; 95%CI 0.4–4.9%) individuals had antibodies against Bovine Herpesvirus type 1 (BoHV-1) and Bovine Viral Diarrhea Virus (BVDV), respectively. Both Parainflueza Virus type 3 (PIV-3) and Bovine Adenovirus (BAV) specific antibodies were detected in 25 out of 66 (37.9%; 95%CI 27.1–49.4) European bison tested. Moreover, the antibodies against Bovine Respiratory Syncytial Virus (BRSV) were present in 10 out of 66 (15.2%; 95%CI 8.4–25.7) animals tested. The seropositivity to PIV-3 was associated with the age, gender, location and health status (healthy/fallen/eliminated) of the animal. For the presence of BAV antibodies, location, year of sampling, population type and health status, were significantly associated factors. Additionally, the PIV-3 and BAV infections were correlated as the presence of BAV antibodies increased the chance of detecting seropositivity to PIV-3 almost nine fold. The work describes exposure to BAV and BRSV in European bison for the first time. The research is being continued in order to understand the impact of those infections on health and survival of this protected ruminant species.

**Key words:** European bison, serology, BoHV-1, BVDV, PIV-3, BAV, BRSV

## Introduction

Infections of European bison should be considered from two perspectives: as endemic threats from putative pathogens infecting this species or/and cross-species transmission between wild and domestic ruminants. Viruses such as BoHV-1,

BVDV, PIV-3, BAV, BRSV play a key role in a multifactorial syndrome called Bovine Respiratory Disease (BRD) which occurs in young cattle, and therefore causes large losses in livestock all over the world. The pathogens damage the lungs and immunosuppress the animal, allowing for secondary agents such as *Manheimia haemolitica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* infect and cause disease, and in severe cases even death (Bednarek *et al.* 2012; Lekeux 2008; Socha *et. al*. 2013). Additionally, BoHV-1 and BVDV are involved in a reproductive losses which needs to be monitored especially in the endangered species such as European bison. Some of the viruses have been investigated in European and American bison *(Bison bison)* previously (Borchers *et al*. 2002; Citterio *et al*. 2003; Frölich *et al.* 1998; Kita *et al.* 1991; Rypuła *et al*., 2011; Salwa *et al*. 2007; Sausker *et al*. 2002; Taulescu *et al*. 2011; Taylor *et al*. 1997; Zarnke *et al*. 1990). Latently or persistently infected with BoHV-1 and BVDV animals may be the reservoirs of the virus spreading the infection in the herd, and transmitting it to other susceptible species including farm animals. Monitoring of the distribution of infections enables to identify the potential threats to European bison health and protect the species at the individual, herd and population levels.

## Study area

Tested animals originated from Białowieża Primeval Forest (67 free-living and 20 captive), Smardzewice (17 captive), Bieszczady region (7 free-living and 5 captive), Pszczyna (11 captive), Borecka Forest (8 free-living), Gołuchów (5 captive), Warsaw ZOO (4 captive), Strzelinko (1 captive).

## Materials and methods

Samples of serum, whole blood or fluids from body cavities were collected during selective eliminations, chemical immobilizations or from dead animals between 2013 and 2014.

All ELISA tests were conducted and calculated according to manufacturer's protocols. For the detection of PIV-3, BAV and BRSV antibodies IDEXX Trivalent Antibody Test kit was used. The samples with S/P (Corrected Sample to Positive percentage) greater or equal to 20% were considered positive. To detect BoHV-1 and BVDV antibodies, IDEXX IBR gB blocking Ab Test and IDEXX BVDV Ab Test were used. The test use a cut-off values of S/N=55% and S/P=0.3, respectively.

Average values were defined as apparent prevalence (AP). The 95% confidence intervals (CI) were determined for binomial distribution of values 0 – seronegative result and 1 – seropositive according to Wald statistics. The analysis assumed probability value significance at $p<0.05$.To estimate the true prevalence (TP)

from AP, Rogan and Gladen (1978) algorithm was used from the Epitools online software (AusVet Animal Health Services). The sensitivity and specificity values of the ELISAs used in the calculation of TP derived from the manufacturer brochures (except for BAV for which 100% was adopted). STATA/IC 13.0 software was used for the statistical analysis of associations. The dependence between the percentage of seropositive bison and the year of sampling, age, sex, location, health status and population type (free-living/captive), was evaluated using univariate logistic regression with determination of the odds ratio (OR) values. In the analysis two variables determining the age of the animals were used: a) discontinuous variable in years (0–24); b) ordinal variable in age groups (group I: calves of 0–1 years, group II: young bison at the age of 2–3 years, group III: adult bison above 4 years).

## Results

The summary of descriptive statistics is presented in Table 1. Only one animal out of 145 tested (0.7%) which originated from Białowieża had BoHV-1 antibodies. The BVDV apparent prevalence was also very low reaching 1.4% as only two animals from 144 tested were found seropositive. Nevertheless, the seroprevalences of PIV-3, BAV and BRSV were much higher and reached the values of 37.9% for the first two pathogens and 15.2% for BRSV. The percentage of seropositive for PIV-3 and BAV European bison was associated with the place of origin of the animal with OR values equal to 0.7 (95%CI 0.6–0.9; $p=0.01$) and 0.6 (95% CI 0 0.5–0. 8; $p=0.01$), respectively. The seroprevalence of PIV-3, BAV and BRSV were significantly higher ($p<0.05$) in the European bison population from Białowieża than from other locations. The associations between animal and sampling details and researched virus seroprevalences were summarized in Table 2. Due to the low seroprevalence of BVDV and BoHV-1, these data was omitted from the analysis. The highest percentages of PIV-3 seropositive European bison was found among adult and females. The level of PIV-3 infection in fallen or selectively culled due to health status European bison was also significantly higher compared to the potentially healthy animals that were immobilized for transport or diagnostic purposes. BAV seropositivity was significantly related also to the health status with the highest values among fallen and culled bison. Interestingly, the risk of acquiring BAV antibodies was 1.5 times lower in captive in respect to free-living bison.

A significant positive linear correlation between the age and the percentage of PIV-3 and BAV seropositive European bison was observed, whereas such relationship was not found for other infections, in particular those characterized by a larger seroprevalence such as BRSV (Fig. 1).

**Table 1.** Seroprevalence of Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Parainfluenza Virus (PIV-3), Bovine Adenovirus (BAV) and Bovine Respiratory Syncytial Virus (BRSV) in European bison according to the place of their origin.

| | | Location | Białowieża Primeval Forest | Other | Total |
|---|---|---|---|---|---|
| Seroprevalence | BoHV-1 | n/N[a] | 1/87 | 0/57 | 1/145 |
| | | AP[b] 95%CI | 1.1 0.2–6.2 | 0 | 0.7 0.1–3.8 |
| | | TP[c] 95%CI | 1.0 0–3.2 | 0 | 0.5 0–1.8 |
| | BVDV | n/N[a] | 1/87 | 1/57 | 2/144 |
| | | AP[b] 95%CI | 1.1 0.2–6.2 | 1.7 –1.7–5.3 | 1.4 0.4–4.9 |
| | | TP[c] 95%CI | 0.7 0–3 | 1.3 –0.2–4.9 | 0.9 0–2.9 |
| | PI-3 | n/N[a] | 18/25 | 7/41 | 25/66 |
| | | AP[b] 95%CI | 72.0 52.4–85.7 | 17.1 5.0–29.1 | 37.9 27.1–49.9 |
| | | TP[c] 95%CI | 63.5 39.7–87.3 | 15.2 3.3–27.1 | 17.4 1.6–33.2 |
| | BAV | n/N[a] | 18/25 | 7/41 | 25/66 |
| | | AP[b] 95%CI | 72.0 52.4–85.7 | 17.1 5.0–29.1 | 37.9 27.1–49.9 |
| | | TP[c] 95%CI | 72.0 54.4–89.6 | 17.1 8.5–31.3 | 37.9 26.2–49.6 |
| | BRSV | n/N[a] | 7/25 | 3/41 | 10/66 |
| | | AP[b] 95%CI | 28.0 14.3–47.6 | 7.3 –1.0–15.6 | 15.2 8.4–25.7 |
| | | TP[c] 95%CI | 32.2 12–52.4 | 8.4 –0.8–17.6 | 17.4 7.5–27.4 |

n/N number of seropositive animals/total number of animals tested;

AP apparent seroprevalence;

TP estimated true seroprevalence

**Table 2.** The associations between PIV-3, BAV and BRSV seropositivities in European bison and year of sampling, age group, gender, population (free-living; captive) and health status (healthy – chemically immobilized; fallen; selectively eliminated).

| Association of seropositivity to | n/N | AP[a] | TP[b] | OR[c] | 95%CI[d] | *p* |
|---|---|---|---|---|---|---|
| **PIV-3 with** | | | | | | |
| Age group*: | | | | | 6.8 | 0.01 |
| I (0–1 years) | 1/7 | 14.3 | <0 | 1 | – | – |
| II (2–3 years) | 0/13 | 0 | <0 | 1 | – | – |
| III (4 and more years) | 20/38 | 52.6 | 37.4 | 6.6 | 0.7–60.8 | 0.09 |
| Gender*: | | | | | | |
| ♀ | 17/33 | 51.5 | 35.9 | 1 | – | – |
| ♂ | 7/29 | 24.1 | <0 | 0.3 | 0.1–0.9 | 0.03 |
| Population | | | | | | |
| free-living | 13/33 | 39.4 | 19.5 | 1 | – | – |
| captive | 12/33 | 36.4 | 15.4 | 0.9 | 0.3–2.4 | 0.8 |

| Association of seropositivity to | n/N | AP[a] | TP[b] | OR[c] | 95%CI[d] | *p* |
|---|---|---|---|---|---|---|
| Health*: | | | | 13.7 | 3.3–56.4 | >0.001 |
| healthy | 12/50 | 24.0 | 24.3 | 1 | – | – |
| fallen | 5/7 | 71.4 | 72.3 | 7.9 | 1.3–46.2 | 0.02 |
| eliminated | 8/9 | 88.9 | 88.9 | 25.3 | 2.9–223.6 | 0.004 |
| **BAV with** | | | | | | |
| Age group: | | | | 1.8 | 0.7–4.3 | 0.2 |
| I (0–1 years) | 2/7 | 28.6 | 28.6 | 1 | – | – |
| II (2–3 years) | 2/13 | 15.4 | 15.4 | 0.45 | 0.05–4.2 | 0.5 |
| III (4 and more years) | 16/38 | 42.0 | 42.0 | 1.8 | 0.3–10.6 | 0.5 |
| Gender | | | | | | |
| ♀ | 13/33 | 39.4 | 39.4 | 1 | – | – |
| ♂ | 11/29 | 37.8 | 37.8 | 0.9 | 0.3–2.6 | 0.9 |
| Population* | | | | | | |
| free-living | 18/33 | 54.5 | 54.5 | 1 | – | – |
| captive | 7/33 | 21.2 | 21.2 | –1.5 | –2.5–0.4 | 0.007 |
| Health* | | | | 5.7 | 1.7–19.2 | 0.006 |
| healthy | 14/50 | 28.0 | 28.0 | 1 | – | – |
| fallen | 5/7 | 71.4 | 71.4 | 6.4 | 1.1–37.1 | 0.04 |
| eliminated | 6/9 | 66.7 | 66.7 | 5.1 | 1.1–23.4 | 0.03 |
| **BRSV with** | | | | | | |
| Age group | | | | 1.2 | 0.4–4.0 | 0.7 |
| I (0–1 years) | 0/7 | 0 | <0 | 1 | – | – |
| II (2–3 years) | 3/13 | 23.1 | 26.6 | 1.9 | – | 0.9 |
| III (4 and more years) | 5/38 | 13.2 | 15.2 | 0.9 | – | 0.9 |
| Gender | | | | | | |
| ♀ | 6/33 | 18.2 | 21.0 | 1 | – | – |
| ♂ | 3/29 | 10.3 | 11.9 | 0.5 | 0.1–2.3 | 0.3 |
| Population | | | | | | |
| free-living | 3/33 | 8.1 | 9.4 | 1 | – | – |
| captive | 7/33 | 19.0 | 21.8 | 2.7 | 0.6–11.5 | 0.2 |
| Health | | | | 1.4 | 0.3–6.3 | 0.6 |
| healthy | 7/50 | 14.0 | 16.2 | 1 | – | – |
| fallen | 1/7 | 14.3 | 16.5 | 1 | 0.1–9.8 | 1.0 |
| eliminated | 2/9 | 22.2 | 25.6 | 1.7 | 0.3–10.2 | 0.532 |

* statistically significant differences between groups ($p < 0.05$);

AP apparent seroprevalence;

TP estimated true seroprevalence (Rogan, Gladen, 1978);

OR odds ratio;

95%CI 95% confidence interval.

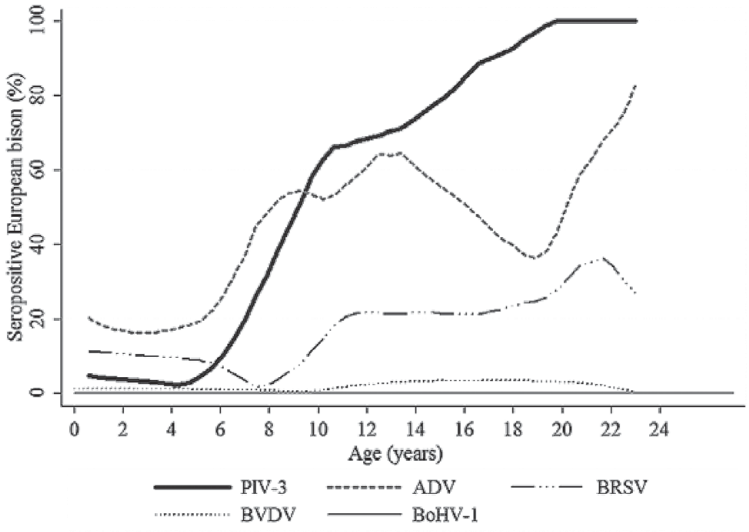


**Fig. 1.** The proportion of seropositive European bison in relation to age presented as smoothed polynomial lines (LOESS).

## Discussion:

The serosurvey is a non-invasive way of estimating possible contact between the animal and the pathogen. It is not synonymous with occurrence of clinical disease, however gives an indication of the epidemiological threats. Infections with the investigated viral bovine respiratory pathogens in free-ranging ungulates rarely lead to the development of symptoms of disease (Borchers *et al.* 2002; Kita *et al.* 1991; Rypuła *et al.* 2011; Salwa *et al.* 2007; Taylor *et al.* 1997; Zarnke *et al.* 1990). The most common pathological lesions in European bison population in Białowieża Primeval Forest in years 2008–2013 were changes in respiratory system resulting in pneumonia (45.3%) and emphysema (32.9%) (Krzysiak *et al.* 2014). The studied pathogens involved in BRD in cattle, focuses mostly in young animals causing high mortality in calves. Krasińska and Krasiński (2004) described that the percentage of fallen European bison did not differ significantly between the age groups, except for the calves which showed the highest percentage of 15.2% in 1974–2002. The seroprevalence of PIV-3, BAV and BRSV was rather lower in younger animals and increased with the age, which may suggest similar susceptibility to the infection in all age groups. The increasing with age seroprevalence which is often observed in infectious diseases is connected to the increasing number of possible infection events.

## BVDV and BoHV-1

European bison infected with BVDV-1 were showing clinical signs of depression, fever, lack of appetite, diarrhea, hypersalivation, dyspnea and possible mortality after 24 hours (Taulescu *et al*. 2011). In British ZOO, some oryx positive for BVDV antibodies at the time of sampling were found in poor condition and had intermittent diarrhea. In the same ZOO, a BHV-1 antibody positive European bison had erosive inflammation of the respiratory and gastrointestinal tract which has lead to its death (Frölich *et al.* 1998). Another pestivirus (BDV-4), similar to BVDV-1 has decimated the population of Pyrenean chamois (*Rupicapra pyrenaica*), causing high mortality and reproductive losses in pregnant females (Arnal *et al*. 2004). BVDV can affect American bison (55.3%) (Sausker *et al*. 2002). The seroprevalence of BVDV in Polish cattle is high. In cattle, the symptoms include respiratory tract insufficiency and diarrhea (in young calves may lead to death) as well as persistently infected (PI) individuals spreading the virus in a herd. The BVDV infection rate studied previously in Polish European bison varied between studies. Only low or no antibody levels were detected between 1999 and 2000 and in 2011, respectively in free-living European bison in Poland (Borchers *et al*. 2002; Rypuła *et al*. 2011). Salwa *et al*. (2007) have shown that 29.5% of European bison were BVDV seropositive in the study in 1991–2001. Such different results may be due to the use of different testing methods such as microneutralisation test, ELISA (full viral antigen or p80 protein) and serum neutralization test. Additionally, the numbers of tested animals was quite limited which made reliable statistics impossible. According to Rypuła *et al*. (2008) BoHV-1 seroprevalence in Polish diary cattle is high. Earlier studies of Rola *et al*. (1999) showed lower BoHV-1 seroprevalence (20%), however it varied between regions. BoHV-1 and other close related alpha-herpesviruses like BoHV-2, CerHV-1 (Cervidae Herpesvirus-3 ), RanHV-1 (Rangifrine Herpesvirus-1) antibodies were detected in multiple species of free-ranging and farmed ungulates. In American bison, BoHV-1 antibodies were reported at the level 38% and 0.3% in free-ranging (Taylor *et al*. 1997; Zarnke *et al.* 1990); 43.8% in ranch-raised animals (Sausker *et al*. 2002). In Poland, no BoHV-1 antibodies were found in the European bison most recently studied by Salwa *et al.* (2007) and Rypuła *et al.* (2011). Earlier, the seroprevalence at the level of 13.3% was reported in European bison by Kita *et al*. (1991). BoHV-1 was suspected to be involved in the etiology of balanoposthitis of Polish European bison, however the fact has never been confirmed (Borchers *et al*. 2002). Transmission of BoHV-1 to the population may result in reproductive losses and therefore the infection should be monitored in order to identify and eliminate latently infected animals, being the reservoirs of the pathogen in the population. In the present study, low seroprevalence of BVDV and BoHV-1 indicates that those infections are not endemic in the European bison in Poland. However, any direct or indirect contact with domestic ruminants may result in a fast spread of the disease

as the bison are susceptible to those infections. Therefore, testing for BVDV and BoHV-1 should be continued in order to avoid such future health problems to this endangered species.

## PIV-3, BAV and BRSV

PIV-3 antibodies are detected in almost all Polish dairy herds. Their seroprevalence at the level of 50% is observed in approx. $^2/_3$ of herds (Rypuła *et al*. 2008). The presence of PIV-3 antibodies was reported in 36% and up to 100% of free-ranging American bison (Taylor *et al*. 1997; Zarnke *et al.* 1990). In 2007 study, PIV-3 antibodies were detected in 13.9% of European bison from Białowieża Primeval Forest (Salwa *et al*. 2007) what is much lower than our result (72%). This difference may be explained by different methods used for diagnosis. Salwa *et al*. (2007) have used hem agglutination inhibition test.

The adenovirus adds up to the clinical picture of BRD, however the prevalence of the virus in Polish cattle has not been estimated. In Finland, for example the seroprevalence of bovine adenovirus type 3 and 7 (BAV-3 and BAV-7) reached 83 and 100% of cattle herds with BRDC (Härtel *et al*. 2004). In ungulates in Turkish ZOO BAV-1 and BAV-3 antibodies were detected in 46.6%, 60.1% of animals, respectively (Yeşilbağ *et al.* 2011). Our study is the first one to report adenovirus infections in wild ruminants including European bison in Poland. It is not clear whether the high seroprevalence (37.9%) could be associated with some health problems and should be investigated. Previous studies has shown that an endotheliotropic adenovirus related to adenovirus haemorragic disease in white-tail deer fawn (AHDV) caused lesions similar to those caused by bluetongue (BT) virus or epizootic hemorrhagic disease (EHD) virus such as pulmonary edema and haemorrhagic entheropathy (Woods *et al.* 1999). Sequence analysis of the genome fragment confirmed that this novel virus is closely related to bovine adenovirus-3 however the biologic properties of these two viruses are clearly distinct (Lapointe *et al.* 1999).

The last virus seroprevalence investigated was the BRSV. The pathogen alone may cause devastating respiratory disease. Between 2000 and 2001 an outbreak of respiratory disease had place in Italy in alpine chamois *(Rupicapra rupicabra)*. Almost 200 animals were found dead with signs of severe fibrinous lobar pneumonia or catarrhal bronchopneumonia. In carcasses and serum BRSV was detected (Citterio *et al.* 2003). In Poland BRSV antibodies were detected in 61.4% of dairy cattle and its prevalence increased with age (Socha *et al.* 2013). There have not been any previous studies on BRSV seroprevalence in European bison in Poland. BRSV antibodies were detected in 31% and 55.3% of ranched American bison (Tailor *et al.* 1997; Sauskers *et al.* 2013). The seroprevalence of 21% was reported in Alpine chamois (Citterio *et al.* 2003) which was comparable to the seroprevalence found among European bison from Białowieża (28%).

The study presents recent epizootic situation of selected viral pathogens among European bison from eight locations in Poland. Possibility of some infections as BAV and BRSV was presented in this species for the first time.

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**Serologiczne badania w kierunku wirusowych zakażeń układu oddechowego u żubrów w Polsce**

**Streszczenie:** Przebadano serologicznie żubry *(Bison bonasus)* na obecność przeciwciał skierowanych przeciwko wirusom wywołującym zaburzenia ze strony układu oddechowego leżące u podstaw tzw. zespołu oddechowego bydła (BRD). Celem badań była ocena potencjalnych zagrożeń dla zdrowia populacji żubrów w Polsce, jak również określenie zależności między infekcjami wirusowymi a wiekiem zwierząt, płcią, typem populacji (wolno żyjące, zagrodowe), geograficznym pochodzeniem, statusem zdrowotnym (padłe, eliminowane immobilizowane) i rokiem pobrania próbki. Żubry pochodzące z ośmiu różnych populacji: Puszczy Białowieskiej (87 zwierząt), Smardzewic (17), Bieszczad (12), Pszczyny (11), Puszczy Boreckiej (8), Gołuchowa (5), warszawskiego ZOO (4) oraz Strzelinka (1) zostały przebadane odpowiednimi komercyjnymi ELISA przeznaczonymi dla bydła. Próbki surowicy, osocza lub płynu pobranego pośmiertnie z jam ciała pochodziły od osobników immobilizowanych do celów diagnostycznych, poddanych badaniu sekcyjnemu odstrzelonych selekcyjnie lub padłych żubrów. Tylko jeden ze 145 (0,7%; 95%CI 0,1–3,8) i dwa z 144 (1,4%; 95%CI 0,4–4,9%) żubrów posiadały przeciwciała odpowiednio przeciw bydlęcemu herpeswirusowi BoHV-1 i wirusowi wirusowej biegunki bydła BVDV. Przeciwciała dla bydlęcego adenowirusa BAV oraz wirusa parainfluenzy PIV-3 wykryto u 25 z 66 (37,9%; 95% CI 27,1–49,9) badanych żubrów. Oprócz tego, przeciwciała skierowane przeciwko syncytialnemu wirusowi oddechowemu bydła BRSV zidentyfikowano u 10 z 66 (15,2%; 95%CI 8,4–25,7) badanych zwierząt. Stwierdzono występowanie zależności między obecnością przeciwciał dla PIV-3 a wiekiem, płcią, pochodzeniem oraz statusem zdrowotnym zwierząt (zdrowe/padłe/wyeliminowane). Istotnymi czynnikami związanymi z seroprewalencją BAV było pochodzenie, rok badania, typ populacji i status zdrowotny. Ponadto zaobserwowano dodatnią korelację między zakażeniem PIV-3 i BAV. Obecność przeciwciał dla BAV zwiększała prawie dziewięciokrotnie szansę wykrycia przeciwciał dla PIV-3. Praca ta opisuje po raz pierwszy wykrycie przeciwciał dla BAV i BRSV u żubrów. Badania te będą kontynuowane w celu poznania wpływu tych zakażeń na zdrowie i przeżywalność tego zagrożonego gatunku przeżuwaczy.