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Serological Study of Exposure to Selected Arthropod-Borne Pathogens in European Bison (*Bison bonasus*) in Poland

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Summary

Bison bonasus is an indigenous species of Central and Eastern Europe with the largest wild population inhabiting Białowieża Primeval Forest; however, free-living and captive European bison are reared in many countries around the world. Despite that the European bison was rescued from the extinction after the First World War, it remains as endangered species. Changing environment as well as human activity may have contributed to the observed increase of the risk of the emergence and re-emergence of pathogens. The aim of the survey was to establish the distribution of four pathogens transmitted by arthropods including three arboviruses [Bluetongue disease virus (BTV), Epizootic haemorrhagic disease virus (EHDV) and Schmallenberg virus (SBV)] and a bacteria (*Francisella tularensis)* in the main populations of European bison in Poland. A total of 251 European bison originating from eight main populations were included in the study and sampled between February 2011 and December 2014. Serum samples originated from chemically immobilized, eliminated or dead by natural causes animals. Additionally, 65 cervids from Białowieża Forest were tested to compare the seroprevalences of other ruminants inhabiting the same environment. The antibodies to SBV and BTV were found in 76.1% and 24.7% of European bison, respectively. In autumn 2012, simultaneous emergence of SBV and BTV in European bison was observed; however, while SBV has spread in all populations scattered around the country, BTV infections were observed only in the north-eastern part of Poland, where BTV cases have been previously reported in domestic ruminants. European bison age was found to be the only significant risk factor for SBV and BTV seroprevalences; however, this association was connected to the animal size, rather than to the length of exposure. None of the animals tested positive for antibodies against EHDV or *F. tularensis*. SBV exposure rate of cervids was much lower (35.4%) than in European bison, while BTV seroprevalence was comparable in both groups.

Introduction

The European bison (*Bison bonasus*), also known as wisent, is the largest terrestrial animal of Europe. In Poland, the species originated from 12 founding animals after its extinction from the wild after the First World War. Since then, significant efforts have been made to conserve the species. Currently, the world population of European bison is over 5000 individuals (Raczyński and Bołbot, 2011-2014). In contrast to the more numerous American species (*Bison bison*) living in the plains and prairies, the majority of wild European bison inhabit thick forests like the Białowieża Primeval Forest, which is located on the border between Poland and Belarus. Nowadays,

Białowieża's European bison population is the largest in the world; however, free-living and captive animals are reared in many European counties around the world. Due to their close relationship to domestic cattle (*Bos taurus*), bison may be susceptible to infectious diseases of domesticated livestock. Similarly, European bison may also be exposed to infectious diseases of wildlife in their natural habitat. The first reports of infectious diseases in European bison refer to infections with *Pasteurella multocida* and *Mycoplasma mycoides* (Wróblewski, 1927). Foot-and-mouth disease (FMD) affected the Polish population in 1940s and 1950s causing high mortality rates in the restored European bison herd (Kita and Anusz, 2006). New epidemiological threats have appeared in the late 20th century. Tuberculosis was diagnosed in the species for the first time twenty years ago, and it is still considered as the most threatening disease to the *B. bonasus*. Despite culling of infected herds and taking all the necessary measures to prevent the spread of the disease according to 40-year national TB eradication and control programme, tuberculosis remains endemic in some European bison populations like Bieszczady (Krajewska et al., 2015).

Climate change, intensification of animal production and globalization may contribute to the increased risk of the emergence of new pathogens or the unexpected appearance of pathogens in other geographic areas (MacLachlan and Guthrie, 2010). Due to the spillover effect, infections of Bluetongue disease virus (BTV), Epizootic haemorrhagic disease virus (EHDV) or the newly discovered Schmallenberg virus (SBV) affecting domestic ruminants may also threat wildlife including endangered species such as European bison. BTV and EHDV are both *Orbiviruses* transmitted by *Culicoides* midges, and both are represented by a number of distinct serotypes with varying virulence (MacLachlan and Guthrie, 2010; Savini et al., 2011). These orbiviruses can cause virus-mediated vascular injury, which can result in mild-to-fatal haemorrhagic clinical disease in infected animals. Bluetongue is an OIE-listed disease of global importance with significant financial implications in ruminants. Prior to 2006, BTV was detected only in the southern regions of the Europe. However, novel BTV serotype 8 has emerged and spread rapidly across the continent. It became evident that BTV was able to be transmitted by apparently new Palaearctic vector species such as *Culicoides obsoletus*, *C. scoticus*, *C. chiopterus*, *C. dewulfi* and *C. pulicaris* resulting in the spread of further BTV serotypes in Europe (MacLachlan and Guthrie, 2010). EHDV infections are known to cause high mortality in white-tailed deer in North America. EHDV also affects domestic and wild ruminants in Africa, Australia and Japan. The disease has only been reported in neighbouring European regions such as Turkey, Israel and Morocco, where EHDV-6 and EHDV-7 strains have been detected (Temizel et al., 2009;

Albayrak et al., 2010; Savini et al., 2011). The susceptibility of cattle to Turkish EHDV-6 has been demonstrated (Batten et al., 2011). Despite the subclinical course of the infection, bovids may play an important role in a hypothetical EHDV emergence in Europe (Maclachlan et al., 2015). In addition, the most abundant in Poland midge species of *C. punctatus* and *C. obsoletus* have been reported to transmit EHDV in North America and Japan (Savini et al., 2011).

In 2011, a new arbovirus – Schmallenberg virus (SBV) emerged in Europe which caused milk drop and diarrhoea in adult cows and congenital malformations in newborn calves and lambs. SBV belonging to *Orthobunyavirus* genus is transmitted by the same midge species as BTV. The virus has spread across Europe faster than BTV affecting a larger panel of species including most European free-living ruminants (Larska et al., 2014b; Rossi et al., 2015).

Tularaemia is a serious zoonosis caused by *Francisella tularensis*. This gram-negative bacterium contains several highly pathogenic subspecies, whose distribution is pervasive in the Northern Hemisphere (Ellis et al., 2002; Hestvik et al., 2015). *Francisella tularensis* subsp. *holarctica* remains the main endemic threat in Europe. The disease is a notifiable bacterial zoonosis in Poland due to its high pathogenic potential. The pathogen has a complicated epidemiological cycle, including involvement of wild animal species and arthropod vectors such as ticks. *F. tularensis* has been isolated from more than 250 species, including mammals, invertebrates, birds, amphibians and fishes (Friend, 2006; Mörner and Addison, 2008; OIE, 2008). In species susceptible to *F. tularensis*, serology is of limited value as infected animals usually die before they are able to develop detectable antibodies. European bison as representation of the bovine species appear to be a relatively resistant to *F. tularensis* infection (Mörner and Sandstedt, 1983; Feldman, 2003; OIE, 2008).

The aim of the present survey was to establish the distribution of four pathogens transmitted by arthropods including three arboviruses (EHDV, BTV, SBV) and a bacteria (*Francisella tularensis)* in the main populations of European bison in Poland.

Materials and methods

Sample collection

A total of 251 European bison from eight locations [Białowieża Primeval Forest – 52°42′56.396″N 23°55′24.298″E (*n* = 112); Bieszczady Mountains – 49°11′16.404″N 22°40′59.242″E (*n* = 14); Gołuchów – 51°51′5.303″N 17°55′58.04″E (*n* = 5); Niepołomice – 49°59′36.671″N 20°17′56.332″E (*n* = 27); Pszczyna – 49°59′49.312″N 18°58′22.835″E (*n* = 47); Borecka Forest – 54°5′9.395″N 21°55′25.856″E (*n* = 10); Smardzewice –
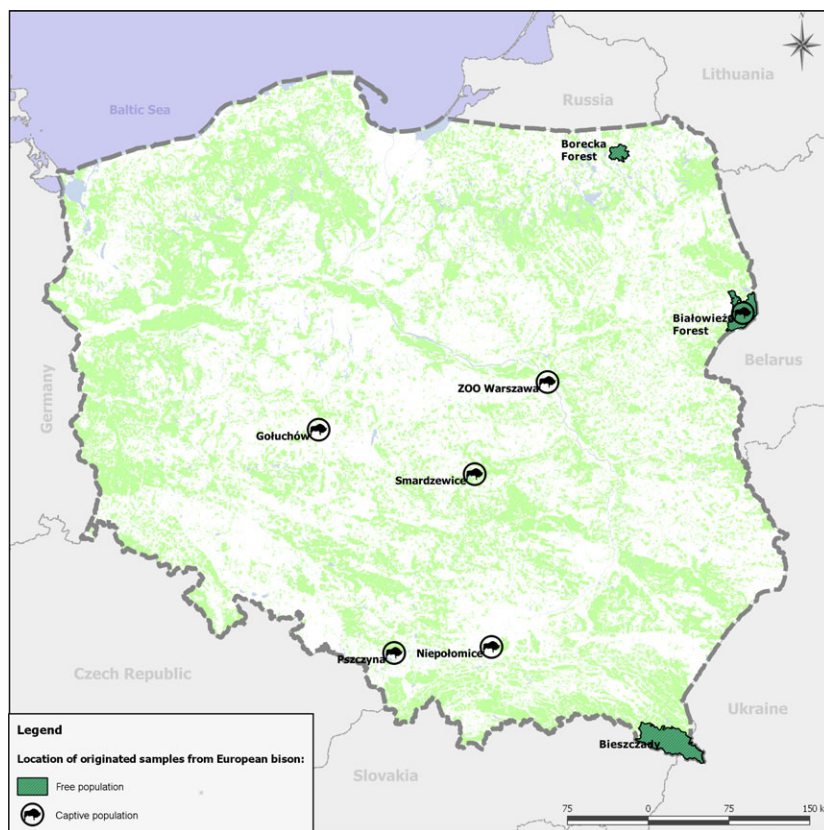
Fig. 1. Map of Poland with the distribution of European bison populations included in the study.

51°28′33.388″N 20°1′47.099″E ($n = 32$); Warsaw ZOO – 52°15′24.177″N 21°1′14.394″E ($n = 4$)], which represent the main populations of European bison in Poland were included in the study and sampled between February 2011 and December 2014 (Fig. 1). All animals, excluding those from Białowieża Forest, were sampled from February 2013.

Eighty-six free-ranging European bison were sampled from three locations; Białowieża Forest ($n = 71$), Bieszczady Mountains ($n = 7$) and Borecka Forest ($n = 8$). The remaining European bison ($n = 165$) were captive animals reared in the fenced reserves. Serum samples were collected from these animals as follows: after chemical immobilization (Krzysiak and Larska, 2014) ($n = 172$), following natural ($n = 35$) or accidental ($n = 2$) deaths and elimination ($n = 42$). The age of the animals ranged between few months to 27 years, with the mean value of 6.6 years (95% CI: 5.8; 7.4). For further statistical analysis, the animals were divided into three age categories using the key of Krasińska and Krasiński (2007) as follows: I – calves ≤1 year of age ($n = 47$); II – young animals between 2 and 3 years of age ($n = 61$); and III – adult animals ≥4 years of age which reached sexual maturity ($n = 133$). The blood was collected into sterile tubes with clot activator for serum separation from the jugular vein, heart or body cavities in dead animals and from the jugular vein in the immobilized bison. The poor quality samples were excluded prior the study for

ensuring the high reliability of results. Additionally, 65 cervids including red deer (*Cervus elaphus*) ($n = 58$), roe deer (*Capreolus capreolus*) ($n = 4$) and elk (*Alces alces*) ($n = 3$) from Białowieża Forest were blood sampled. The cervid samples were collected from animals found dead or killed in accordance with the appropriate Polish legislation (Ministry of Environment, 2005) during the winter hunting season of 2013 and 2014.

Serological tests

The ID Screen ELISA Schmallenberg virus Competition Multi-species (ID.vet, Grabels, France) was used to detect antibodies against SBV nucleoprotein. Selected SBV-seropositive serum samples were confirmed by virus neutralization test described previously (Larska et al., 2014b). Bluetongue IgG or IgM antibodies against VP7 protein were tested using ID Screen Bluetongue Early detection test (ID.vet, Montpellier, France). This ELISA test has high specificity and sensibility for all BTV serotypes and is dedicated to test multiple species, also wild ruminants as declared by the manufacturer and described by some authors (Niedbalski, 2011; Tavernier et al., 2015). The cross-neutralization analysis using a panel of BTV serotypes performed by the reference laboratory (Pirbright Institute) confirmed the specificity of BTV ELISA results as discussed

further. A blocking LSIVet™ Ruminant EHDV Serum ELISA Kit (Live Technologies, Carlsbad, California) was used to test for antibodies against EHD VP7 protein antigen. The test detects antibodies to all 9 existing EHDV serotypes, it does not cross-react with BTV-specific antibodies and is specifically designed to test bovine, cervids and wild ruminant serum samples (Cêtre-Sossah et al., 2014; Daly et al., 2015). All ELISA tests were performed and calculated according to manufacturer's instructions. For detecting antibodies against *F. tularensis*, the serum agglutination test (SAT) using the commercial antigen (Becton Dickinson) was used. The test was carried out as a microagglutination, in accordance with manufacturer's instructions. In brief, 2-fold dilutions of each tested serum sample from 1 : 10 to 1 : 160 were tested. In each plate, the following controls: negative cattle serum, commercial positive *F. tularensis* antiserum (Becton Dickinson) and antigen control were included. The reaction was considered positive at dilutions of 1 : 40 or higher.

Statistical analysis

Statistical analysis was performed using STATA v. 13.0 software (StataCorp LP, Texas, USA). The associations between the geographic location, gender, age group, population type (free-ranging/captive), sanitary status [healthy (anesthetized), eliminated due to their poor health condition), fallen or other (dead in traffic accident)] and Bluetongue and Schmallenberg virus seroprevalences were estimated using chi-squared test and odds ratio (OR) calculated by univariate logistic regression. The cross-correlations for all variables were assessed by Spearman rank test. The generalized linear mixed models (GLMMs) using binomial error structure and logit link function were developed by backward elimination of insignificant (with $P > 0.05$) variables (year of sampling, origin, population type, age, gender, sanitary status) one-by-one. The multicollinearity assessed by variance inflation factor (VIF $\geq$ 10) and Spearman rank test ($\rho > |0.8|$; $P < 0.05$) between the variables was considered when building up the multivariate model. Possible confounding and clustering was analysed as described by Dohoo et al. (2010). To account for clustering, models including random intercept were assessed by checking the variance of the component and other covariates. The model with akaike information criterion (AIC) and bayesian information criterion (BIC) lowest and highest values, respectively, was considered better fitting.

Results

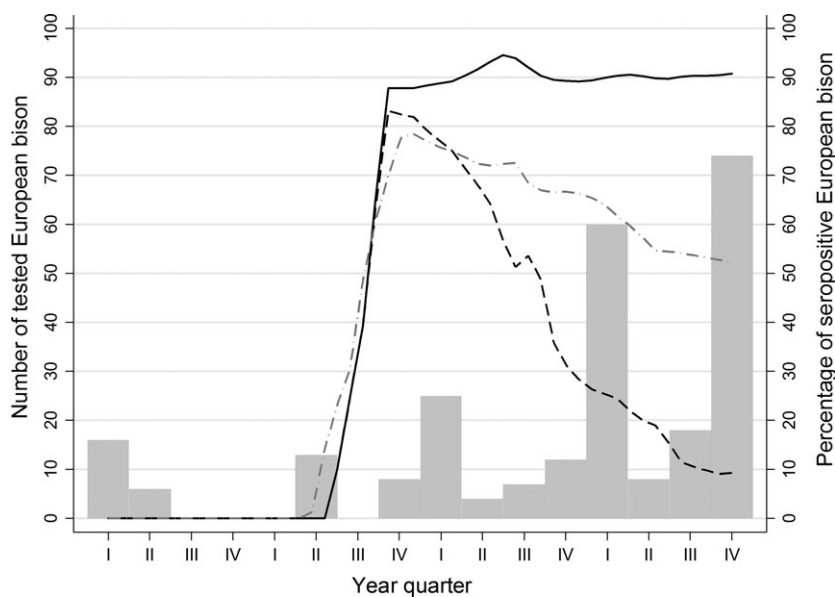### SBV seroprevalence in European bison

In total, 191 of 251 (76.1%, 95%CI: 72.3, 83.1) European bison tested positive for SBV antibodies. Two sera gave

doubtful results. The first seropositive animal was detected in October 2012 in Białowieża Forest. The overall seroprevalence of SBV increased rapidly. None of 6 tested European bison had SBV antibodies in June 2012, while SBV antibodies were detected already in 7 of 8 (87.5%) animals sampled between October and December 2012. The dynamics in the overall SBV seroprevalence are presented quarterly in Fig. 2. SBV seroprevalence in European bison maintained at high-level starting from the fourth quarter (October–December) of 2012 throughout the studied period. In all tested *B. bonasus*, SBV seroprevalence increased significantly ($\chi^2 = 114.8$; $P < 0.001$) by year, from 0% (0/22) in 2011, through 33.3% (7/21) and 89.6% (43/48) in 2012 and 2013, respectively, and up to 90.6% (141/160) in 2014. In the largest European bison population in Białowieża, the yearly seroprevalences [0% (0/22), 35.0% (7/20), 85.7% (30/35) and 80.0% (28/35) in 2011, 2012, 2013 and 2014, respectively] were also significantly different ($\chi^2 = 52.7$; $P < 0.001$). At the same time, SBV seroprevalence in the youngest animals up to 1 year of age decreased from 83.3% (5/6) in 2013 to 51.5% (17/33) in 2014 in all studied populations. None of the tested calves had SBV antibodies in 2012. In Białowieża Forest population, 4 of 5 calves were seropositive in 2013, while only 2 of 9 in 2014.

The virus has spread in the majority of European bison (Table 1) in all studied populations (Fig. 1). Among 216 tested European bison, which could have been at risk of SBV exposure since October 2012 onwards, 191 (88.4%; 95%CI: 83.4, 92.3) had detectable antibodies. The age of European bison was associated with SBV seroprevalence with the highest proportion in the animals older than 1 year (Table 1). The relation is also illustrated in Fig. S1. The borderline significant association between SBV seroprevalence and gender (Table 1) has not been proven for the adult European bison ($\geq$4 years), with the percentages of approx. 94% for both females and males. The type of population (captive/free-ranging) and so-called sanitary status (describing whether the animal was potentially healthy immobilized for diagnostic purposes, selectively eliminated due to their poor body condition or found dead) did not influence SBV seroprevalence.

The multivariable model was developed. No collinearity (Table S1), confounding or clustering of data were observed. In the final GLMM model ($n =$ developed using all the data of potentially exposed from October 2012 onwards European bison), age was confirmed as the only predictor of SBV seropositivity. The risk of SBV-seropositive result increased with age: odds ratios (ORs) between age groups I and II and age groups I and III were 40.2 ($P < 0.001$) and 11.9 ($P < 0.001$), respectively.

Fig. 2. Schmallenberg virus (SBV) and blue-tongue virus (BTV) seroprevalences in Polish European bison by year quarters. The curves indicate local polynomial smoothed lines of total percentages of SBV-seropositive (solid black line) and BTV-seropositive animals (dashed black line) and the percentage of BTV seropositive in three European bison populations (Białowieża National Park, Bieszczady Mountains and Borecka Forest) where BTV infection was confirmed (dot-dashed grey line). Corresponding axis is on the right side. The grey bars present the numbers of animals tested and the values correspond to the axis on the left side.

BTV seroprevalence in European bison

BTV antibodies were found in 62 of 251 (24.7%; 95%CI: 19.3, 30.1) animals. Seropositive *B. bonasus* originated only from Białowieża Forest, Bieszczady Mountains and Borecka Forest from which 57/112 (50.9%), 3/14 (21.4%) and 2/10 (13.3%), respectively, had specific antibodies. The detection of BTV antibodies in European bison from Białowieża Forest coincided with detection of SBV antibodies in October 2012 (Fig. 2). However, the overall quarterly prevalence increased only at the end of 2012 and begun to decrease in the next years (Fig. 2). The decline was larger when only those three populations where BTV antibodies were detected were considered. The yearly seroprevalences in the three populations where BTV infection was confirmed were 35% (7/20), 72.7% (32/44) and 48.9% (23/47) in 2012, 2013 and 2014, respectively. In the European bison from Białowieża, SBV seroprevalences increased significantly ($\chi^2 = 40.3$; $P < 0.001$) from 0% (0/22) in 2011, through 35% (7/20) in 2012 to 82.9% (29/35) in 2013, and decreased to 60% (21/35) in 2014.

Similar to SBV, BTV seropositivity was associated with age of European bison with the lowest seroprevalences found in the youngest animals less than 1 year of age (Table 2, Fig. S1). The BTV seroprevalence was also higher in females; however, the difference was not significant. Inversely, a difference between European bison females (17/62; 27.4%) and males (25/53; 47.2%) was observed ($\chi^2 = 4.8$; $P = 0.03$) when animals 4 years and older were considered. Moreover, the seroprevalence decreased from 60% (3/5) in 2013 to 0% (0/9) in 2014 for the European bison calves.

In the univariable analysis, BTV seropositivity was connected to the sanitary status with the highest proportion in the dead or eliminated due to their poor body condition of European bison (Table 2). However, this relation was not observed in the multivariable model. The risk of BTV seropositivity was associated only with European bison age in the final GLM model ($n = 90$). Similarly to SBV, the risk of BTV-seropositive result increased significantly in group II (OR = 24.9; $P = 0.001$) and III (OR = 18.2; $P < 0.001$) in comparison to group I. As some clustering of data at the origin was observed, the random effect for origin trough maximum likelihood with adaptive Gauss–Hermite approximation was fitted (variance for origin was 1.8; 95% CI: 0.3, 11.8).

In the three populations exposed to both viruses from October 2012, BTV and SBV seropositivities were interrelated ($\chi^2 = 4.3$; $P = 0.04$) with 57 of 102 (55.9%) European bison having antibodies against both pathogens. The presence of antibodies against one pathogen increased 3.3 times ($P = 0.04$) the risk of finding antibodies to the second pathogen when tested by logistic regression.

EHDV and *F. tularensis* seroprevalence in European bison

None of the animals tested positive for antibodies against EHDV or *F. tularensis*.

Cervids from Białowieża Forest

Of 65 cervids originating from Białowieża Forest, 23 (35.4%; 95% CI: 23.4, 47.3) and 14 (21.5%; 95% CI: 11.3, 31.8) animals were seropositive for SBV and BTV,

Table 1. SBV seroprevalences according to different characteristics of European bison and their associations according to the univariable statistical analysis by chi-squared (χ^2) test and odds ratio (OR) in the logistic regression. The data includes populations in which SBV antibodies were detected starting from October 2012 (estimated date of first exposure to the virus)

Parameter	SBV seroprevalence			χ^2	P[e]	OR[f]	P[e]
	n/N[a]	%[b]	95%CI[c,d]				
Origin (n[g] = 216)							
Białowieża Primeval Forest	65/78 [h]	83.3	73.2–90.8	15.0	0.035	–	0.13
Bieszczady Mountains	14/14	100	76.8–100				
Gołuchów	3/5	60	14.7–94.7				
Niepołomice	27/27	100	87.2–100				
Pszczyna	40/46	87	73.7–95				
Borecka Forest	9/10	90	55.4–99.7				
Smardzewice	31/32	96.9	83.8–100				
Warsaw Zoo	4/4	100	39.8–100				
Population type (n = 216)							
Captive	132/148	89.2	83.0–93.7	0.01	0.9	–	0.9
Free-living	61/68	89.7	80.0–95.8				
Gender (n = 212)							
Female	105/110	95.4	89.7–98.5	9.4	0.002	1	0.04
Male	84/102	82.3	73.5–89.2			0.2	
Age group (n = 207)							
I (≤1 year)	25/41	61.0	44.5–75.8	40.7	>0.001	1	
II (2–3 years)	51/52	98.1	89.7–99.9			32.6	0.001
III (≥4 years)	108/114	94.7	88.9–98.0			11.5	>0.001
Sanitary status (n = 216)							
Healthy (anesthetized)	142/159	89.3	83.4–93.6	4.1	0.25	–	0.4
Eliminated	39/42	92.9	80.5–98.5				
Fallen	11/13	84.6	54.5–98.1				
Other[i]	1/2	50.0	1.2–98.7				

[a]Number seropositive/all tested.
[b]Percentage.
[c]95% confidence interval of the percentage.
[d]For the values of 0% and 100% one-sided test 97.5%CI.
[e]P value < 0.05 was considered significant.
[f]Odds ratio of logistic regression.
[g]Number of observations in analysis without missing data.
[h]Two inconclusive results.
[i]Dead in traffic accident.

respectively. Thirty-nine per cent (95% CI: 24.0, 56.6) and 29.6% (95% CI:13.7, 50.1) of cervids sampled in the winter of 2013 (n = 38) and 2014 (n = 27), respectively, had SBV antibodies. The same proportions were as follows: 36.8% (95%CI: 21.8, 54.0) and 0% for the BTV-seropositive cervids.

Similar to the European bison, no cervid tested positive for EHDV or *F. tularensis* antibodies.

Discussion

The threat of emerging and re-emerging arthropod-borne diseases in Europe has been increasing over the recent years (MacLachlan and Guthrie, 2010). Northern European *Culicoides* species are becoming competent vectors of BTV and EHDV as well as SBV allowing these viruses to spread from

tropical or subtropical ecosystems. The emergence of these diseases is of great importance especially in endangered species such as European bison. In addition, monitoring of infectious diseases in wild animals is needed to effectively manage and protect farm animals. Serological monitoring in domestic and wild ruminants, monitoring of vector activity and the presence of infectious agents in the insects are important as an early warning system of discussed diseases. Despite that the number of European bison included in this study may seem limited, almost 18% of Polish population (251 of estimated 1400 animals) was tested for the presence of SBV, BTV, EHDV and *F. tularensis* antibodies.

The infection with a newly emerged Culicoides-borne SBV in wild ruminants in Poland was first confirmed in a fallen elk from Białowieża Forest in December 2012 (Larska et al., 2013). More recent study showed 27.7% overall

Table 2. BTV seroprevalences according to different characteristics of European bison and their associations according to the univariable statistical analysis by chi-squared (χ^2) test and odds ratio (OR) in the logistic regression. The data includes three populations (BNP, Bieszczady Mountains and Borecka Forest) in which SBV antibodies were detected starting from October 2012 (estimated date of first exposure to the virus)

Parameter	BTV seroprevalence			χ^2	P [e]	OR [f]	P [e]
	n/N [a]	% [b]	95%CI [c,d]				
Origin (n [g] = 99)							
Białowieża Primeval Forest	57/77	74.0	62.8–83.4	19.3	<0.001	1	
Bieszczady Mountains	3/12	25.0	5.5–57.2			0.1	0.003
Borecka Forest	2/8	20.0	2.5–55.6			0.1	0.003
Population type (n = 99)							
Captive	18/32	56.3	37.7–73.6	0.8	0.4	–	0.4
Free-living	44/67	65.7	53.1–76.8				
Gender (n = 95)							
Female	27/38	71.0	54.1–84.6	0.9	0.3	–	0.3
Male	35/57	61.4	47.6–74.0				
Age group (n = 90)							
I (≤1 year)	3/17	17.6	3.8–43.4	18.8	<0.001	1	
II (2–3 years)	12/16	75.0	47.6–92.7			14	0.002
III (≥4 years)	42/57	73.7	60.3–84.5			13	<0.001
Sanitary status (n = 99)							
Healthy (anesthetized)	18/43	41.8	27.0–57.9	15.0	0.002	1	
Eliminated	33/41	80.5	65.1–91.1			5.7	<0.001
Fallen	9/13	69.2	38.6–91.0			3.1	0.09
Other [h]	2/2	100	15.8–100			1	–

[a]Number seropositive/all tested.
[b]Percentage.
[c]95% confidence interval of the percentage.
[d]For the values of 0% and 100% one-sided test 97.5%CI.
[e]P value < 0.05 was considered significant.
[f]Odds ratio of logistic regression.
[g]Number of observations in analysis without missing data.
[h]Dead in traffic accident.

seroprevalence in free-living ruminants in Poland at the end of 2013 (Larska et al., 2014b). The proportion of SBV-seropositive animals in the few European bison included in the study was significantly higher (81.8%) in comparison with wild cervids (30.6% in red deer and 22.6% in roe deer). SBV infections have also been reported in European bison in Western Europe at the beginning of virus epidemic (EFSA, 2014). The virus was isolated from a *B. bonasus* aborted foetus in Germany (ProMED-mail, 2012), suggesting a possible clinical outcome of the SBV infection in the species. First detection of SBV antibodies in European bison at the end of 2012 corresponds with the transmission of the virus to livestock and wildlife in Poland (Larska et al., 2014a,b). A sharp increase of SBV seroprevalence in European bison in winter 2012/2013 depicts the wave of infection that affected naïve populations, which is characteristic for the emerging diseases (Afonso et al., 2014). A similar situation was observed in the cervids in Belgium in 2011 (Linden et al., 2012). SBV has spread evenly in the European bison populations included in the study and infected most of the tested animals, which were presumably

exposed to it since October 2012. The overall SBV seroprevalence in all *B. bonasus* populations was higher than the seroprevalences observed in wild cervids in Białowieża Primeval Forest in this study and in the whole country (Larska et al., 2014b), which may be connected to the differences in species susceptibility or the size of animal. European bison is the largest native mammal in the continent. As the infection rates in all studied European bison populations were higher than in the domestic or wild ruminants from corresponding regions, the species may be suspected as an important reservoir of SBV in Poland. Additionally, European bison often live in the environments preferred by *Culicoides* that provide the midges with favourable conditions for reproduction. The wallows created by American bison and filled with water and waste are preferentially colonized by *C. sonorensis* and *C. variipennis*, North American vectors of such arboviruses as BTV and EHDV in (Pfannenstiel and Ruder, 2015). In case of domestic ruminants, SBV seroprevalence was also higher in cattle than in small ruminants (Larska et al., 2014a), which may be explained by the lower dose of SBV necessary to produce an infection in

bovids comparing to sheep, which would need a 10-fold virus dose to seroconvert (Wernike et al., 2012; Poskin et al., 2014). Furthermore, larger hosts excrete more carbon dioxide and 1-octen-3-ol, the volatile attractants for haematophagous midges, which may increase the risk of the exposure to vector-borne pathogens (Koch and Axtell, 1979; Zimmer et al., 2015). As shown by Viennet et al. (2013), midges prefer to feed on the largest hosts available in the environment. The vector preferences may explain the significant differences in the SBV seroprevalence between European bison calves and older animals. An alternative explanation could be the increased risk of the exposure to the circulating virus over the time. As SBV antibodies may persist for at least 2 years after the exposure in cattle (Elbers et al., 2014), the high SBV seroprevalence observed in an adult European bison may be the sum of infections that occurred at different times. Nevertheless, demonstrating the presence of antibodies in the European bison under the age of 1 year may be used to predict the dynamics of an epizootic disease. In European bison, most calvings occur between May and July (Krasińska and Krasiński, 2007), and therefore, the calves may be immediately exposed to active midge vectors. The non-invasive placenta in ruminants do not allow most antibodies to pass, and thus, the presence of specific antibodies in the calves may be due to the uptake of colostrum from the immune mother or an infection. Although no data are available on the duration of SBV colostral immunity in European bison or cervids, we can assume by analogy to cattle that the maternal antibodies are present during first 5–6 months of life (Elbers et al., 2014). Decline in the percentage of SBV-seropositive yearlings from 2013 to 2014 suggests lower exposure of those animals to the virus indicating a decrease in SBV circulation. Similar dynamics of SBV infections was also observed in wild ruminants in Germany with the decrease of SBV-seropositive animals in 2013/2014 hunting season (EFSA, 2014; Mouchantat et al., 2015). Only three of 12 (25%) European bison born in 2014 had SBV antibodies; these results are likely to be due to passive immunity rather than an acute infection, especially considering the high seroprevalence rates in their mothers.

The highest estimated impact of SBV infection is associated with the reproductive losses. In SBV-infected herds, congenital deformities resulting from SBV uterine infections are estimated to be 8%, 3% and 2% in lambs, calves and goat kids, respectively (Dominguez et al., 2014). Little is known about the intrauterine infections in wild ruminants. SBV RNA has not been detected in the cervid foetuses in Belgium (Linden et al., 2012) or in Spain (Fernández-Aguilar et al., 2014). As aborted foetuses and dead newborns in wild animals are extremely difficult to sample due to the scavenging and difficulties in locating them, the ability of SBV to cause foetus abnormalities or

abortions in those animals remains unknown. Two cases of SBV infection in American bison neonates in Germany and France have been described (EFSA, 2013). In our studies, five European bison foetuses collected at Białowieża Primeval Forest and Smardzewice had negative antibody ELISA and real-time RT-PCR results (Larska, unpublished data). The analysis of the numbers of newborn European bison registered between 2011 and 2014 in the pedigree book (Raczyński and Bołbot, 2011-2014) has not shown any unusual alterations in reproductive performance in the populations.

As described by other authors (Rossi et al., 2015), the spread of BTV in the Polish European bison population with respect to SBV has been slower, in spite of the fact that both viruses appeared simultaneously using the same midge vector in the autumn 2012. BTV-seropositive animals were found only among three European bison populations in the eastern part of Poland suggesting eastbound transmission of the pathogen. This is the first report describing BTV infection in wildlife in this region of Europe. The Polish serotype was determined as BTV-14 in 2012 (European Commission, 2012). The same virus has been declared circulating by the Pirbright Institute in UK, in Lithuania, Latvia, Estonia in 2012 and in Russia 2 years prior (Panferova et al., 2012). BTV-14 infection was confirmed in four European bison from Białowieża Primeval Forest and in cattle in the neighbouring region of Sokółka by real-time RT-PCR at the Pirbright Institute (Krzysiak, unpublished data; Pirbright Institute, 2012). The same homologous to South African vaccine BTV-14 strain virus has been observed circulating in this area between 2011 and 2014 (Orłowska et al., 2016). The virus was also isolated in KC4 (*C. sonorensis*-derived cell line) and BHK1 (baby hamster kidney) cells (http://www.reoviridae.org/dsrna_virus_proteins/reoid/btv-14.htm). The presence of BTV-14 antibodies has been also reported in free-living European bison in Russia (Pavlova et al., 2013). No clinical signs of BTV-14 have been observed, and the close similarity of the strains isolated in the north-eastern Europe suggests the spread of an attenuated vaccine-like strain in the field. The emergence of BTV-14 in the north-eastern Europe should be distinguished from BTV-8 epidemic in Western Europe which began in 2006 as they are probably two separate, unconnected events. BTV-8 infections have not been detected in Poland. Until now, infection with BTV serotype 1, 4 and 8 have been confirmed in domestic and wild ruminants in Europe (García-Bocanegra et al., 2011; Ruiz-Fonsa et al., 2014). European bison have been shown to be susceptible to natural and experimental infection with BTV previously (Ludwig and Silinski, 2008; Sanderson, 2010; Tessaro and Clavijo, 2001). The severity of the infection is usually BTV serotype-dependent; however, as in case of FMD, European bison may be more susceptible to BTV infection in

comparison with other species (Kaandorp-Huber, 2008). During the 2006 European BTV-8 outbreak, the morbidity and mortality rates in zoo-kept European bison reached 40% and 20%, respectively, and were the highest rates reported when compared to other species (Sanderson, 2010). Fatal cases of BTV-8 infection in European bison have been described in the Netherlands and Germany (Glunz, 2008; Kaandorp-Huber, 2008; Ludwig and Silinski, 2008). In 2008, BTV-8 outbreaks in European bison from Hardehausen and Hanau were reported and despite intensive clinical treatment most animals died (Glunz, 2008). The clinical signs included depression, lesions of oral mucosa, corneal enema, respiratory symptoms, difficulties in swallowing, drooling and lameness due to coronitis. On the other hand, the American bison remained unaffected after experimental BTV-11 infection, despite seroconversion and viremia which lasted for 4 weeks (Tessaro and Clavijo, 2001). Polish BTV-14 which appears to be similar to the South African vaccine strain has been shown to produce only mild clinical signs in the experimentally exposed sheep (Flannery et al., 2014).

The higher BTV seroprevalence in older European bison, especially in the adult males may be explained by the size of the animal which increases the exposure rate to the insect vector (Koch and Axtell, 1979; Viennet et al., 2013; Zimmer et al., 2015). European bison males by 6 years of age reach the average weight of approx. 750 kg (up to 920 kg) whereas females can be almost 40% lighter (Krasińska and Krasiński, 2007). The differences in BTV seroprevalence in adult males and females might be also related to species biology (Krasińska and Krasiński, 2007) as reported for BTV-8 infections in red deer in France (Rossi et al., 2015). For most of the year, males are managed separately or within so-called bachelor groups consisting of 2–3 adult males. They join the mixed groups of females and calves in oestrus and during winter concentration. The gathering of larger herds in winter does not appear to be of importance for the transmission of Culicoides-borne diseases (CBD) as the activity of the midges reduces to zero below 9°C (EFSA, 2014). However, male bison may be exposed to different midge subpopulations as they dwell on larger acreages and are often seen grazing the pastures of neighbouring farmland. No statistical differences in the distances made by adult European bison males and females were observed after monitoring by telemetric collars, which may be due to the fact that most of data were collected during the cold months (November–April) (Kowalczyk et al., 2013). However, it was proven that the males move significantly further away from the forest comparing to the females. Besides the sex dimorphism manifested particularly in body mass and the space usage behaviour, the BTV seroprevalence was influenced by the age of the European bison. Similar to SBV, it may

be explained again by the increasing size of the animal and the extended time of exposure to the virus. The association between BTV seropositivity and age of the animal has been described also for red deer (García-Bocanegra et al., 2011; Rossi et al., 2015). It was proven that BTV can circulate independently from domestic transmission cycle and can be found in free-living cervid population even where a rigorous BTV vaccination programme in livestock is implemented. Similar to red deer, which seem to be a natural BTV reservoir, European bison may be responsible for the maintenance of the virus in the sylvatic cycle and possible spill-back events may be expected when the infection or vaccination-induced antibodies decrease in the susceptible livestock (Lorca-Oró et al., 2014).

The absence of EHDV infection in European bison was not surprising, as the pathogen has been never reported in this part of Europe. However, the experiences drawn from the re-emergence of BTV, SBV and recent African swine fever (Pejsak et al., 2014) in this region demonstrates that the risk of diseases previously regarded as exotic increases. EHDV outbreaks in cattle have been reported in European Union neighbouring countries. In 2006, EHDV-7 emerged in Israel with significant losses in cattle similar to Ibaraki disease (Yadin et al., 2008). EHD outbreaks in North America usually cause significant mortality in white-tailed deer, while other species such as American bison become infected accidentally and show no signs of the disease (Nol et al., 2010). However, the recent EHDV epidemic in U.S. in 2012 has shown that the morbidity can reach 7% in bison, which may be considered a significant issue for the endangered species (Stevens et al., 2015).

Wild animals are good environmental markers for many infectious diseases (Al Dahouk et al., 2005; Szulowski et al., 2015). Serological surveys can be conducted with success on the relatively resistant wild species such as wild ungulates and wild carnivores (OIE, 2008; Hestvik et al., 2015; Szulowski et al., 2015). The investigation of the European bison for tularaemia has never been performed in Poland. Furthermore, there is no data available regarding this issue in Europe and even in the world. The potential source of *F. tularensis* is contaminated meat and water (Anda et al., 2001; Hestvik et al., 2015); however, the evolvement of arthropods in the transmission of the bacteria has been also confirmed (Svensson et al., 2009; Reis et al., 2011; Gehringer et al., 2013). Ticks are probably the main vector of *F. tularensis* in Poland (Formińska et al., 2015). The absence of the antibodies to *F. tularensis* may suggest that the species is not susceptible to the infection. Another pathogen, which is transmitted similarly, tick-borne encephalitis virus (TBEV) was also absent in the European bison from Białowieża Primeval Forest, despite the fact that TBEV infections are endemic in the area (Biernat and Karbowiak, 2014). However, the epizootic situation of the

disease is unknown as only cases of human exposure were reported. In the last 15 years, only 56 human cases of tularaemia have been diagnosed in the country; however, this number is likely to be underestimated. According to the annual reports published by the National Institute of Public Health – National Institute of Hygiene (2001-2015), cases of the tularaemia infections seem to be scattered throughout the country and there is no special region in Poland for the occurrence of the disease. Thus, this is the first report of serosurvey conducted in the animals in the country.

In conclusion, the spread of SBV has been confirmed in all European bison populations included in the study, while BTV infections were observed mostly in the north-eastern part of Poland where BTV cases have been previously reported in domestic ruminants. The absence of EHDV in Polish European bison was expected, as no transmission in this part of Europe has ever been reported. However, EHDV emergence is potentially possible as the competent vector is in place. Although our results were negative for *F. tularensis* antibodies, the potential role of European bison as TBE reservoir is not unlikely and should be investigated further. The epidemiological threats to European bison should be constantly monitored in order to protect this endangered species from further extinction.

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Supporting Information

Additional Supporting Information may be found in the
online version of this article:

Table S1. Variance inflation factors (VIF), coefficients of
determination (R^2) Spearman correlation matrix between
all the variables including data gathered in the study since
October 2012 ($N = 207$).

Fig. S1. Locally weighted scatter plot smoothing (LOW-
ESS) curve representing the association between the pro-
portion of seropositive animals (SBV – solid black line;
BTV – dashed grey line) and age of European bison
exposed to the infections from October 2012. The BTV data
included only the three European bison populations where
specific antibodies were detected.