

Article

Learn the Past and Present to Teach the Future—Role of Active Surveillance of Exposure to Endemic and Emerging Viruses in the Approach of European Bison Health Protection

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Abstract: As the population size of free-living European bison in Poland has increased significantly over the last few years, conservation and management should be reconsidered to face new challenges, which aim to protect the whole population, not just individuals. The aim of our study was to analyse prevalence and identify risk factors for infections with viruses affecting reproduction (BVDV, BoHV-1, and BoHV-4), causing respiratory diseases (BRV3, BAdV-3, and BRSV), and emerging vector-borne viruses (BTV and SBV). Over 600 serum samples used in serology and 270 lung tissue samples, and 284 nasal swabs used in virological tests were collected from 24 free-living and captive European bison herds between 2016 and 2023. The seroprevalences varied between low (0.3–6.1% for BVDV, BoHV-1, and BoHV-4), medium (15.9% for BRSV and 22.1% for BTV) and high (43.3% for BRV3, 59.5% for BAdV-3, and 69.5% for SBV). Interspecies' and intraspecies' risk of endemic viral infections was observed to be density-dependent, while the effect of anthropogenic factors on the spread of infections in free-living European bison has not been demonstrated. BTV is no longer circulating, while SBV has become endemic. The higher exposure to viruses in the European bison eliminated on the basis of a veterinary decision indicates the importance of an expert supervision for disease control and prevention.

Keywords: European bison; surveillance; infection ecology



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1. Introduction

The European bison (*Bison bonasus*) is the largest land mammal in Europe, which was rescued from total extinction in the wild just after the First World War. This year, the anniversary of 100 years of restitution will be celebrated to commemorate the undertaking to start the recovery of the species from the remaining specimens scattered in enclosures throughout Europe. In addition to the limited gene pool (the species has been recovered only from 12 individuals, including only seven as founders of the Lowland line—LB), the main threats to the species involve epidemiological threats. As the Polish population has reached close to 2500 individuals, which is over one fourth of the *Bison bonasus* world population [1] and its status has changed to 'Near Threatened' on the IUCN Red List [2], conservation and management should be reconsidered to face new challenges, which aim

to protect the whole population, not just individuals. In the last few years, the total population size of free-living European bison in Poland has increased significantly (Table S1, Figure S1). Additionally, the overall number of European bison kept in captivity is decreasing (Table S1, Figure S2), in favour of the establishment of new free-living subpopulations. The average population growth of European bison is approx. 7.5% per year; however, some free-living populations, such as the Bieszczady and Zachodniopomorskie herds, has doubled or increased by 85%, respectively, in the last seven years (Table S1). The historical documentation prepared by Dr. Wróblewski on the health threats of the European bison in the last remaining population in the Białowieża Forest at the beginning of the XIX century included common diseases transmitted from cattle [3]. One hundred and ten years later, the number of European bison inhabiting the Polish part of Białowieża Forest reached the former population size (Table S1) [4] (pp. 92–93). Under field observations and analysis of current infectious threats to European bison, a continuous surveillance has been performed by the authors since 2011 (Figure 1) [5].

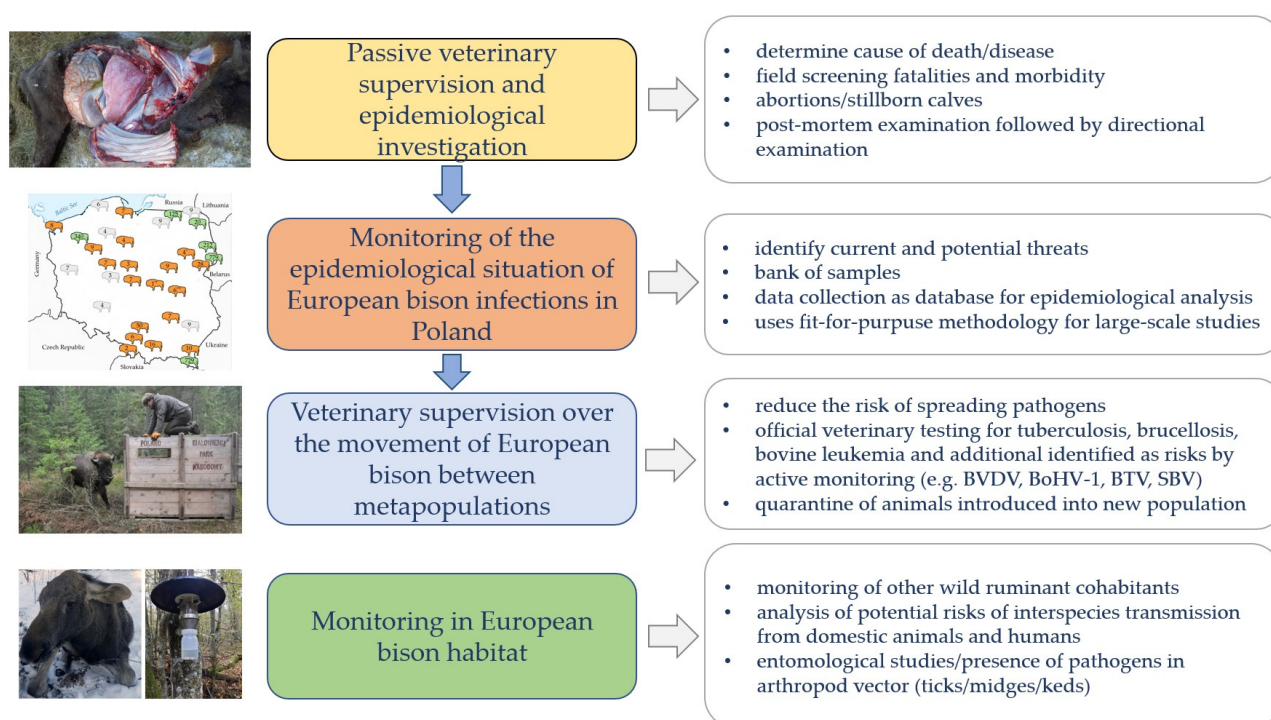


Figure 1. Elements of European bison health monitoring conducted under veterinary supervision in all free-living and captive populations in Poland [5].

As far as viral infections are concerned, we have concentrated firstly on those that can affect reproduction and thus threaten the survival of the population, such as pestiviruses (bovine viral diarrhoea virus—BVDV) and herpesviruses (bovine herpesvirus type 1 and 4—BoHV-1 and BoHV-4). Secondly, we have concentrated on those causing respiratory diseases most commonly diagnosed at the post-mortem examination of European bison [6], such as bovine rhinovirus 3 (BRV3, formerly bovine parainfluenza virus 3—BPIV-3), bovine adenovirus type 3 (BAdV-3), and bovine respiratory syncytial virus (BRSV) [7]. Additionally, the impact of emerging pathogens, such as bluetongue virus (BTV) and Schmallenberg virus (SBV), is being monitored as a model of climate-sensitive infections transmitted through arthropod vectors [8]. Two models of virus transmission should be considered [9,10]. The most common, density-dependent, occurring usually by direct contact via respiratory route, shows a linear correlation between infection/disease prevalence and population density. The frequency-dependent infections are much more difficult to predict and control and include indirect vector-borne, venereal or environmental transmissions.

The premise of this research was to use monitoring data to verify the following hypotheses: (1) the dynamic increase of the European bison population in the recent years increases the risk of interspecies virus transmission between the species and domestic cattle; (2) larger and more dense European bison herds promote a higher virus intraspecies transmission rate; (3) anthropoppression of the European bison habitat influences the exposure to studied viruses; (4) emerging climate-sensitive infections become endemic; and (5) the rationale behind the selection of virus infections for active surveillance is justified by their impact on European bison health and conservation.

2. Materials and Methods

2.1. Study Design

A total of 610 serum samples were collected from European bison from 24 (out of 30 registered in 2021 [1]) different populations including wild-ranging European bison from the Białowieska ($n = 105$), Borecka ($n = 64$), and Knyszyńska ($n = 79$) Forests, Bieszczady ($n = 48$), Zachodniopomorskie ($n = 10$), and Augustowska ($n = 2$) and several other herds kept in captivity, which were sampled between 2016 and 2023 (Figure 2).



Figure 2. Distribution of European bison populations in Poland. The free-living and captive populations, which were included in the study, are marked in green and orange, respectively, while grey represents the remaining European bison herds from which no samples were available. The size of the population is marked as numbers of individuals registered in the latest edition of the European Bison Pedigree Book [1], except for non-existing herds from Kiermusy (* last data from 2020), ZOO Łódź (** 2016), and those depopulated due to tuberculosis in herds from Smardzewice (***) 2017).

The samples originated both from female ($n = 309$) and male ($n = 286$) European bison in the age range between 3 days and 25 years, with 15 and 49 missing records for gender and age, respectively. European bison were sampled solely on the occasion of other procedures and were not immobilized or euthanised for the tests described hereby. Samples were taken from individuals ($n = 384$) that were pharmacologically immobilized, by collars placed with telemetric transmitters, or for diagnostic reasons (e.g., when transported to other populations or suspected of infectious diseases, e.g., tuberculosis); from those that were fallen, meaning, found dead ($n = 58$); from those that were euthanised ($n = 152$) due to poor health, in accordance with the corresponding decisions of the Minister of the Environment and the General Director for Environmental Protection; or from those found dead in traffic (car and train) accidents ($n = 15$). From the immobilized animals, blood was collected through the puncture of the external jugular vein (*vena jugularis externa*) and less often from the tail vein (*vena caudalis mediana*). Blood from the dead, necropsied animals was collected in the form of a clot from the heart or from body cavities, whereas from eliminated animals, blood was also collected from the external jugular vein. Blood was collected into sterile 9 mL tubes, centrifuged within 24 h, while the obtained serum was archived frozen at $-70\text{ }^{\circ}\text{C}$ in the biobank of the Department of Virology, NVRI, until analysis. To calculate the minimal sample size of European bison in each of the populations to substantiate a prevalence of 1% or below with the overall α error set to 0.05, the FreeCalc software of Epitools [11] and the population sizes determined by the European bison Pedigree Books (2016–2021) [1], summarized in Table S1, were used.

For the respiratory virus investigation, 270 lung tissue samples and 284 nasal swabs in universal transport medium (UTM) tubes (Copan, Brescia, Italy) from 467 wisents (231 females, 232 males, and 4 unrecognized) from different regions of Poland were collected for the study. The samples originated from 276 free-living and 191 captive animals. The collected samples of lung tissue were homogenised in PBS to obtain 10% suspension in Lysing matrix D tubes (MP Biomedicals, Illkirch-Graffenstaden, France), using the Fastprep-24 5G system (MP Biomedicals, Santa Ana, CA, USA). Nasal swabs were directly used for the extraction of genetic material. For BTV testing, 66 whole blood samples, collected in EDTA tubes from European bison between 2016 and 2019, were used.

2.2. Serological Testing

The presence of antibodies was tested using ELISA kits dedicated for bovids listed in Table S2. The tests were performed in accordance with the manufacturer's instructions.

2.3. Molecular Testing

DNA and RNA extraction. Viral RNA was extracted from 140 μL of nasal swab samples and lung tissue homogenates using a QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's guidelines. Ribonucleic acid was eluted in 50 μL of elution buffer and stored at $-70\text{ }^{\circ}\text{C}$. Viral DNA was extracted from 200 μL of nasal swab samples using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), eluted in 30 μL of an elution buffer and stored at $-70\text{ }^{\circ}\text{C}$.

Real-time PCRs. Real-time PCRs for BRV3 and BAdVs detection were run using previously described primers (Table S3). The RT-PCR for BRV3 was run in 20 μL of a reaction mix that comprised 11.25 μL of water, 5 μL of the 5 \times QuantiTect Virus Master (Qiagen, Hilden, Germany) Mix, 0.5 μL of each BRV3-specific forward and reverse primer (10 μM), 0.5 μL of a specific probe (10 μM), 0.25 μL of the 100 \times QuantiTect Virus RT Mix (Qiagen), and 2 μL of the RNA sample. After 20 min of reverse transcription at $50\text{ }^{\circ}\text{C}$ and a 7 min incubation at $95\text{ }^{\circ}\text{C}$, 45 cycles of amplification were run, each consisting of 30 s of denaturation at $95\text{ }^{\circ}\text{C}$ and 1 min of annealing/elongation at $57\text{ }^{\circ}\text{C}$. As a positive control, the BRV3 strain Dargen (Friedrich-Loeffler-Institut, FLI, Riems) was used.

Real time PCR for BAdVs was run in 25 μL of a reaction mix that comprised 8 μL of water, 12.5 μL of the 2X Path-IDTM qPCR Master Mix (ThermoFisher Scientific, Waltham, MA, USA), 1 μL of each BAdV-specific forward and reverse primer (10 μM), 0.5 μL of the

BAdV-specific probe (10 μ M), and 2 μ L of the DNA sample. After a 10 min incubation at 95 °C, 47 cycles of amplification were run, each consisting of 15 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 45 s of elongation at 72 °C. The test was validated using the reference strains BAdV-1 B-10; BAdV-2 B-19; BAdV-3 WBR1; BAdV-4 THT/62; BAdV-7 Fukuroi; and BAdV-8 Misk/67 (FLI, Riems) and was confirmed to detect all of them.

BTV detection via real-time RT-PCR was performed using the LSI VetMAX bluetongue Virus NS3—All genotypes kit (Thermo Fisher Scientific, Gent, Belgium), according to the manufacturer's protocol.

All real-time PCR amplifications were performed using a LightCycler 96 Instrument (Roche, Mannheim, Germany).

2.4. Statistical Analysis

The results were presented as apparent seroprevalences because the sensitivities/specificities of the tests used have not been determined for the European bison sera, but only for the bovine (or ovine as is the case of multispecies format of SBV ELISA) sera (Table S2). The univariable associations between the seroprevalences to studied viruses and herd-level variables (origin and year; population type, captive; free-ranging; and population size) and individual-level (age group, gender, and sanitary status) variables were estimated using Fisher's exact test. The free-living and captive populations were categorized on the basis of population size (Table S1) by the 25th and 75th centiles into three groups. The division into age groups was dictated by the generally accepted principle into calves (\leq one year old), juveniles (2–3 years old), and adults (\leq 4 years old) [4] (p. 222). The correlations between all the variables were assessed by Cramér's V or Spearman rank tests. The generalized linear mixed models (GLMMs) were developed by the backward elimination of insignificant (with $p > 0.05$) predictor variables one-by-one. The logit link function was used to model the probability of detection of a seropositive animal. The collinearity (Cramér's V above 0.3; Spearman $\rho > |0.5|$; $p < 0.05$) between the predictors was considered when building up the multivariable model. Possible confounding and clustering were analysed as described by Dohoo et al. [12]. 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. Additionally, the associations between the demographic density number of habitants per km² and the degree of urbanization (DEGURBA; %) based on Central Statistical Office data [13] as variables representing anthropoppression were analysed for the free-living populations, excluding the Zachodniopomorskie herds and the Augustowska Forest as the sample sizes were limited. The Kruskal–Wallis test was used for this purpose. All the analyses were performed using the STATA v.13.0 software (StataCorp LP, TX, USA). The p value ≤ 0.05 was considered significant in all the analyses.

3. Results

3.1. Descriptive Statistics

3.1.1. Viruses Affecting Reproduction

Of the 610 European bison, only 3 (0.5%) were positive and one was doubtful for BVDV-specific antibodies. BVDV seropositive animals included two 3–4-year-old bulls from the free population at the Knyszyńska Forest and one bull from the Białowieska Forest, all eliminated due to poor health conditions. BoHV-1 seropositive animals included only two (0.3%) eliminated 8- and 15-year-old bulls from the Białowieska Forest. Out of 396 European bison tested for BoHV-4 antibodies, 24 (6.1%) reacted positive, while 6 were doubtful in ELISA. The animals with doubtful results were excluded from further analysis. In the univariable analysis (Table 1), the seroprevalences were associated with origin (BoHV-4), population type (BoHV-4), free-living population size (BoHV-1), age (BoHV-4), and sanitary status (BVDV and BoHV-4). In case of BoHV-4, the infection occurred only in some populations, including the Białowieska Forest, where it infected over one fourth of

all free-ranging European bison tested. Interestingly, only adult animals were exposed to the virus, and the infection was more frequent in eliminated ones.

Table 1. Descriptive statistics of European bison exposure to viruses affecting reproduction including bovine viral diarrhoea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), and bovine herpesvirus type 4 (BoHV-4), based on the presence of specific antibodies (serology).

Variable	BVDV Seropositives				BoHV-1 Seropositives				BoHV-4 Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Herd—level												
Origin			0.14	1.0			0.1	1.0			52.2	0.0001
Białowieża Forest	1/105	1.0			2/105	1.9			15/57	26.3		
Borecka Forest	0/64	0			0/64	0			0/47	0		
Knyszyńska Forest	2/79	2.5			0/79	0			5/60	8.3		
Bieszczady	0/48	0			0/48	0			1/22	4.5		
Zachodniopomorskie	0/10	0			0/10	0			-	-		
Augustowska Forest	0/2	0			0/2	0			-	-		
Bałtów	0/7	0			0/7	0			0/7	0		
Białowieża	0/61	0			0/61	0			1/39	2.6		
Muczne (Bieszczady)	0/7	0			0/7	0			0/7	0		
Gołuchów	0/15	0			0/15	0			0/11	0		
Jabłonowo	0/14	0			0/14	0			-	-		
Kiermusy	0/8	0			0/8	0			0/6	0		
Międzyzdroje	0/7	0			0/7	0			0/4	0		
Niepołomice	0/32	0			0/32	0			0/26	0		
Pszczyna Park	0/1	0			0/2	0			0/2	0		
Pszczyna (Jankowice)	0/96	0			0/96	0			2/62	3.2		
Smardzewice	0/29	0			0/29	0			0/26	0		
Szewce	0/1	0			0/1	0			-	-		
Ustroń	0/3	0			0/3	0			0/2	0		
ZOO Bydgoszcz	0/1	0			0/1	0			-	-		
ZOO Gdańsk	0/3	0			0/3	0			0/1	0		
ZOO Łódź	0/4	0			0/4	0			0/4	0		
ZOO Poznań	0/2	0			0/2	0			-	-		
ZOO Warszawa	0/10	0			0/10	0			0/7	0		
Year			3.5	0.8			6.5	0.5			7.9	0.09
2016	0/63	0			0/63	0			856	14.3		
2017	1/92	1.1			0/92	0			3/85	3.5		
2018	1/132	0.8			0/132	0			7/127	5.5		
2019	0/80	0			0/80	0			4/71	5.6		
2020	1/73	1.4			1/74	1.4			2/51	3.9		
2021	0/70	0			1/70	1.4			-	-		
2022	0/92	0			0/92	0			-	-		
2023	0/7	0			0/7	0			-	-		
Population type			3.0	0.09			1.9	0.2			16.2	<0.001
free-living	3/308	1.0			2/308	0.6			21/186	11.3		
captive	0/301	0			0/302	0			3/204	1.5		
Population size—free-living			0.9	0.6			7.6	0.1			8.5	0.01
small (≤ 125)	0/67	0			0/67	0			0/48	0		
medium (144–707)	2/145	0.9			0/145	0			16/110	9.1		
large (≥ 715)	1/96	1.5			2/96	3.1			5/25	11.1		
Population size—captive			-	-			-	-			2.0	0.4
small (≤ 6)	0/39	0			0/40	0			0/31	0		
medium (7–42)	0/198	0			0/198	0			3/124	6.2		
large (≥ 43)	0/64	0			0/64	0			0/49	2.4		

Table 1. Cont.

Variable	BVDV Seropositives				BoHV-1 Seropositives				BoHV-4 Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Animal—level												
Age group			1.2	0.5			1.4	0.5			18.5	<0.001
≤1 year old	0/110	0			0/110	0			0/71	0		
2–3 years old	1/118	0.8			0/119	0			0/82	0		
≥4 years old	1/331	0.3			2/332	0.6			24/212	11.3		
undefined	1/48	2.0			0/48	0			0/25	0		
Gender			0.2	0.6			2.2	0.141			0.6	0.4
female	2/306	0.6			0/306	0			11/203	5.4		
male	1/282	0.3			2/283	0.7			13/176	7.4		
undefined	0/21	0			0/21	0			0/11	0		
Sanitary status			9.0	0.03			6.0	0.11			21.7	<0.001
immobilized (healthy)	0/384	0			0/385	0			10/263	3.8		
eliminated	3/152	2.0			2/152	1.3			10/98	10.2		
fallen	0/58	0			0/58	0			1/22	4.5		
dead in traffic accident	0/15	0			0/15	0			3/7	42.8		

¹ number of seropositive European bison/all tested (*N* = 609 and 610 for BVDV and BoHV-1, respectively; *N* = 390 for BoHV-4), excluding inconclusive samples. Significant differences marked in bold (*p* < 0.05).

3.1.2. Respiratory Viruses

Out of the 378 European bison tested, 164 (43.3%), 225 (59.5%), and 60 (15.9%) tested positive for BRV3, BAdV-3, and BRSV, respectively. The seroprevalence was highly variable among different locations for all three viruses (Table 2). The seroprevalences of all three viruses were significantly higher in free-ranging European bison; however, the seroprevalence increased with the free population size only for BRV3 and with the captive population size for BAdV-3. Surprisingly, BRV3 and BRSV seropositive test results were more frequent in smaller, captive herds. All the seroprevalences were age-dependent and were highest in the eliminated European bison, suggesting clinical implications of the infections. However, the antibody levels used for the semi-quantitative estimation of exposure to virus were distributed evenly in the seropositive samples (Table S4). On this basis, no correlation between the antibody level and age or sanitary status was observed.

Genetic material of BRV3 was detected in the lungs from 19-year-old bulls eliminated due to poor health conditions from the Białowieska Forest, which suffered from later-confirmed pasteurellosis. However, the low viral genome copy numbers unabled further sequencing and strain characterization. BAdV DNA was not detected in any of the nose swab and lung samples. Therefore, whether the viruses circulating in European bison are species specific or are genetically related to bovine strains remains to be solved.

Table 2. Descriptive statistics of European bison exposure to respiratory viruses including bovine rhinovirus 3 (BRV3), bovine adenovirus type 3 (BAdV-3), and bovine respiratory syncytial virus (BRSV), based on the presence of specific antibodies (serology).

Variable	BRV3 Seropositives				BAdV-3 Seropositives				BRSV Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Herd—level												
Origin	178.1 <0.001				82.6 <0.001				90.0 <0.001			
Białowiecka Forest	56/71	78.9			56/71	78.9			22/71	31.0		
Borecka Forest	24/49	49.0			36/49	73.5			12/49	24.5		
Knyszyńska Forest	44/48	91.7			42/48	87.5			3/48	6.2		
Bieszczady	3/30	10			17/30	56.7			2/30	6.7		
Zachodniopomorskie	-	-			-	-			-	-		
Augustowska Forest	2/2	100			1/2	50			2/2	100		
Bałtów	4/6	66.7			4/6	66.7			0/6	0		
Białowieża	7/43	16.3			22/43	51.1			2/43	4.6		
Muczne (Bieszczady)	0/7	0			0/7	0			0/7	0		
Gołuchów	0/10	0			2/10	20			7/10	70		
Jabłonowo	3/9	33.3			2/9	22.2			0/9	0		
Kiermsy	4/8	50			3/8	37.5			3/8	37.5		
Międzyzdroje	1/6	16.7			0/6	0			0/6	0		
Niepołomice	5/16	31.2			6/16	37.5			0/16	0		
Pszczyna Park	0/2	0			1/2	50			0/2	0		
Pszczyna (Jankowice)	1/43	2.3			23/43	53.5			0/43	0		
Smardzewice	7/9	77.8			1/9	11.1			2/9	22.2		
Szewce	1/1	100			0/1	0			1/1	100		
Ustroń	0/3	0			1/3	33.3			0/3	0		
ZOO Bydgoszcz	0/1	0			1/1	100			0/1	0		
ZOO Gdańsk	2/3	66.6			1/3	33.3			0/3	0		
ZOO Łódź	0/1	0			0/1	0			1/1	100		
ZOO Poznań	-	-			-	-			-	-		
ZOO Warszawa	0/10	0			6/10	60.0			3/10	15.3		
Year	0.8 0.9				6.74 0.2				4.4 0.4			
2016	-	-			-	-			-	-		
2017	-	-			-	-			-	-		
2018	57/129	44.2			82/129	63.6			18/129	14.0		
2019	35/80	43.7			42/80	52.5			13/80	16.2		
2020	29/74	39.2			50/74	67.6			14/74	19.0		
2021	31/70	44.3			36/70	51.4			14/70	20.0		
2022	12/25	48.0			15/25	60.0			1/24	4.0		
2023	-	-			-	-			-	-		
Population type	77.1 <0.001				47.9 <0.001				6.8 <0.001			
free-living	129/200	64.5			152/200	76.0			41/200	20.5		
captive	35/178	19.7			73/178	41.0			19/178	10.7		
Population size—free-living	6.3 0.04				0.49 0.8				12.3 0.002			
small (≤ 125)	26/51	51.0			37/51	72.5			14/51	27.4		
medium (144–707)	54/82	65.8			63/82	76.8			7/82	8.5		
large (≥ 715)	49/67	73.1			52/67	77.6			20/67	29.8		
Population size—captive	10.6 0.005				5.9 0.05				25.8 <0.001			
small (≤ 6)	8/36	22.2			10/36	27.8			12/36	33.3		
medium (7–42)	26/100	26.0			40/100	40.0			7/100	7.0		
large (≥ 43)	1/42	2.3			23/42	54.8			0/42	0		

Table 2. Cont.

Variable	BRV3 Seropositives				BAdV-3 Seropositives				BRSV Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Animal—level												
Age group			70.6	<0.001			49.8	<0.001			19.3	<0.001
≤1 year old	5/61	8.2			30/61	49.2			2/61	3.2		
2–3 years old	22/80	27.5			25/80	31.2			7/80	8.8		
≥4 years old	129/204	63.2			153/204	75.0			49/204	24.0		
undefined	8/33	24.2			17/33	51.5			2/33	6.1		
Gender			0.02	0.9		0.006	0.9				0.0007	1.0
female	88/201	43.8			120/201	59.7			32/201	15.9		
male	76/177	42.9			105/177	59.3			28/177	15.8		
Sanitary status			41.6	<0.001			20.7	<0.001			6.3	0.1
immobilized (healthy)	76/242	31.4			127/242	52.5			31/242	12.8		
eliminated	58/85	68.2			67/85	78.8			20/85	23.5		
fallen	22/40	55.0			22/40	55.0			8/40	20.0		
dead in traffic accident	8/11	72.7			9/11	81.8			1/11	9.1		

¹ number of seropositive European bison/total tested (*N* = 378). Significant differences marked in bold (*p* < 0.05).

3.1.3. BTV and SBV—Representatives of Arboviruses

Of the 610 European bison tested, seropositive results for BTV and SBV were obtained for 135 (22.1%) and 424 (69.5%) individuals, respectively. Additionally, 1 and 10 samples, respectively, gave doubtful reactions and were excluded from further analysis. Most of BTV infections (98%) were limited geographically to eastern populations, except for two European bison from Pszczyna and one from ZOO Łódź, while SBV was spread throughout all populations, except for the enclosure in Muczne (Bieszczady) and ZOO Poznań (Table 3). BTV and SBV seroprevalences were higher in free-living populations, and the exposure to SBV further increased with the size of these populations. The exposure to both viruses increased with age. Interestingly, the association between the exposure and sanitary status was significant with lowest BTV seroprevalence in healthy animals and highest SBV seroprevalence of eliminated (suspected of disease) ones.

The overall seroprevalence of BTV was much lower in all populations and in the exposed ones as compared to SBV (Table 3). In the recent years, BTV seroprevalence has decreased, suggesting a decrease in population immunity, while SBV has fluctuated but recently towards a rise (Figure 3). This is most probably connected to the decline or absence of the BTV circulating in the environment. This is confirmed by the lack of BTV antibodies in the sentinel group of calves between 6 months and 1 year of age (*N* = 94) (Figure 3), which are deprived of maternal antibodies and, therefore, are naïve to infection, becoming an indicator of virus circulation in a given year during the season of vector activity. Another argument for the lack of BTV circulating is the negative results for BTV detection in the blood of 66 tested seropositive European bison by RT-PCR. Since BTV may persist in mononuclear blood cells for several months after the infection, the lack of its genetic material suggests an old infection, prior to 2016. No new infections and an antibody decline in previously infected European bison resulted in a general decrease in the herd immunity. In contrast, SBV seroprevalence in sentinel calves fluctuated annually reportedly as in the general population, which indicates an active circulation of SBV in most years, except for 2019 (Figure 3).

Table 3. Descriptive statistics of European bison exposure to the *Culicoides*-borne bluetongue virus (BTV) and Schmallenberg virus (SBV), based on the presence of specific antibodies (serology).

Variable	BTV Seropositives				SBV Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Herd—level								
Origin			220.0	<0.001			79.4	<0.001
Białowieża Forest	69/105	65.7			75/102	73.5		
Borecka Forest	10/64	16.1			36/62	58.1		
Knyszyńska Forest	37/79	46.1			63/78	80.8		
Bieszczady	1/48	2.1			40/47	85.1		
Zachodniopomorskie	0/9	0			8/9	87.5		
Augustowska Forest	0/2	0			2/2	100		
Bałtów	0/7	0			7/7	100		
Białowieża	14/61	23.0			40/61	65.6		
Muczne (Bieszczady)	0/7	0			0/7	0		
Gołuchów	0/15	0			7/15	46.7		
Jabłonowo	0/14	0			5/14	35.7		
Kiermusy	1/8	12.5			5/8	62.5		
Międzyzdroje	0/7	0			1/7	14.3		
Niepołomice	0/32	0			21/32	65.6		
Pszczyna Park	0/2	0			2/2	100		
Pszczyna (Jankowice)	2/96	2.1			68/94	72.3		
Smardzewice	0/29	0			28/29	96.5		
Szewce	0/1	0			1/1	100		
Ustroń	0/3	0			3/3	100		
ZOO Bydgoszcz	0/1	0			1/1	100		
ZOO Gdańsk	0/3	0			2/3	33.3		
ZOO Łódź	1/4	25.0			3/4	25.0		
ZOO Poznań	0/2	0			0/2	0		
ZOO Warszawa	0/10	0			5/10	16.6		
Year			12.7	0.08			43.5	<0.001
2016	21/63	33.3			47/63	74.6		
2017	19/92	21.0			75/91	82.4		
2018	22/132	16.2			98/130	75.4		
2019	21/80	26.9			43/78	55.1		
2020	18/74	24.6			44/73	60.3		
2021	19/70	27.5			35/69	50.7		
2022	15/91	14.7			74/89	82.9		
2023	0/7	0			7/7	100		
Population type		91.2	<0.001				5.0	0.02
free-living	117/307	38.1			224/300	74.6		
captive	18/302	6.0			199/300	66.3		
Population size—free-living			18.6	<0.001			9.4	0.009
small (≤ 125)	11/67	16.4			39/65	60.0		
medium (144–707)	59/144	41.0			112/142	78.9		
large (≥ 715)	47/96	24.0			73/93	78.5		
Population size—captive			1.7	0.4			0.04	0.4
small (≤ 6)	2/40	5.0			26/40	65.0		
medium (7–42)	10/198	5.0			131/197	66.5		
large (≥ 43)	6/64	9.3			42/63	66.7		

Table 3. Cont.

Variable	BTV Seropositives				SBV Seropositives			
	<i>n/N</i> ¹	%	χ^2	<i>p</i>	<i>n/N</i> ¹	%	χ^2	<i>p</i>
Animal—level								
Age group			67.7	<0.001			64.2	<0.001
≤1 year old	7/110	6.4 ²			48/110	43.6		
2–3 years old	5/118	4.3			81/118	68.4		
≥4 years old	116/332	34.9			269/324	83.0		
undefined	7/49	14.2			26/48	54.2		
Gender			1.3	0.2			0.4	0.5
female	66/306	21.6			205/299	68.6		
male	67/282	23.8			204/280	72.8		
undefined	2/21	9.5			15/22	68.2		
Sanitary status			63.4	<0.001			35.9	<0.001
immobilized (healthy)	46/385	11.6			257/379	67.8		
eliminated	60/152	39.3			130/150	86.7		
fallen	23/57	41.8			26/56	45.4		
dead in traffic accident	6/15	40.0			10/15	66.6		

¹ number of seropositive European bison/all tested (*N* = 609 for BTV and 600 for SBV), excluding inconclusive samples. ² All animals were <6 months of age; therefore, the seropositivity is most likely connected to passive immunity, rather than exposure. Significant differences marked in bold (*p* < 0.05).

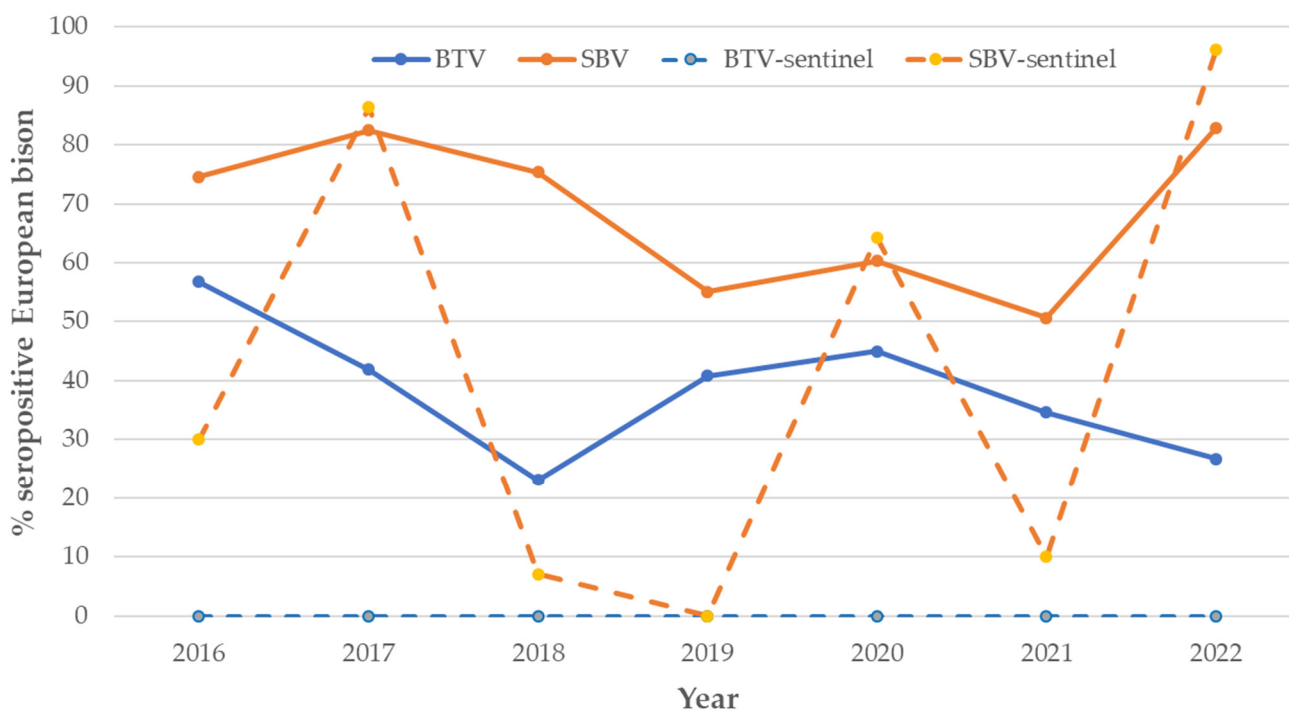


Figure 3. Seroprevalences (percentage of European bison with specific antibodies) to bluetongue virus (BTV) and Schmallenberg virus (SBV) in all animals and in the sentinel group (94 calves between 6 months and 1 year of age, deprived of maternal antibodies). BTV seroprevalences referred to the populations of the eastern part of Poland (Białowieska, Borecka, and Knyszyńska Forests, Bieszczady and Kiermusy), where BTV infections were present (*N* of European bison = 372) as well as the Schmallenberg virus (SBV) in all the populations within the surveillance (*N* = 600), between 2016 and 2022.

3.2. Factors Affecting Potential Interspecies Transmission from Cattle and Intraspecies Transmission among European Bison

The relevant data used to assess potential interspecies spread included exposure to bovine-specific viruses such as BVDV, BoHV-1, and BoHV-4 and higher exposure rates of European bison free-living populations, in particular the larger population size, which have the greatest possibility of approaching farms and coming into contact with cattle. The intraspecies (European bison—European bison) spread was analysed using a density-dependent variable of population size, especially in captive herds.

Therefore, the data analysis provided following observations:

- BVDV and BoHV-1 spread from cattle (reservoir species) to European bison; however, on the basis of the results, the neighbour transmission is sporadic and concerns the largest free-living populations such as the Białowieża Forest (Table 1). Other explanations such as low susceptibility of European bison to BVDV and BoHV-1 infection, low prevalence of the infections in cattle in surrounding farms, and biosecurity/prophylactic measures present in neighbouring cattle farms should be considered.
- Potential direct contact between cattle and European bison was reflected by the presence of specific antibodies BoHV-4, mostly in the largest free-living populations with the highest seroprevalence in the Białowieża Forest (Table 1). The only exceptions were the Pszczyna and Białowieża captive populations, where two and one BoHV-4 seropositive bulls were detected, respectively.
- The higher seroprevalences of respiratory viruses in free-living animals also suggested possible direct spill over from cattle. However, some circulation, in particular of BRV3 and BAdV-3 was observed also in captive herds, which also corroborated independent, intraspecies circulation of these viruses in the European bison population.
- Higher seroprevalences in larger free-living populations for BRV3 and in larger captive populations for BAdV-3 suggested a density-dependent mode of transmission.
- Surprisingly, exposure to BRV3 and BRSV was higher in smaller captive herds such as ZOOs or small show enclosures, which suggested the source of infection within the herd and where European bison have multi-species contacts with other non-domestic animals, including exotic ones. Another possibility may be indirect exposure connected to the passive transmission of pathogens by human staff and visitors of the enclosures.
- Arthropod viruses BTV and SBV were more widely spread in free-ranging European bison; however, since the transmission is vector-dependent, the environmental conditions would be more likely to have an impact, rather than possible direct contact with cattle.
- Highest SBV seroprevalence in the medium and largest free-ranging populations suggests density dependence; however, significant variation in SBV seroprevalence in all and in sentinel (naïve to infection, 6 months—1 year old calves) European bison by year also indicated frequency-dependence.

3.3. Anthropogenic Factors Affecting Virus Transmission

As none of the viruses tested had zoonotic potential, we were only able to test for indirect effects, which potentially could influence European bison special behaviour, habitat loss/fragmentation, pollution, stress, and contact with domestic animals through farming and livestock grazing. Most of the seroprevalences (except for BVDV and BoHV-1) were related to human population density. However, some linearity was observed only for BoHV-4, BTV, and SBV, and the association was negative, meaning human population density decreased the exposure (Table 4). In the case of BoHV-4, this could be connected to the bias towards the predominance of the infection in European bison from the Białowieża and Knyszyńska Forests (Table 1). While, in the case of BTV and SBV, more populated areas may limit the suitable environment for vector breeding, the use of plant protection products such as insecticides or repellents for human and animal protection can reduce their numbers, thus affecting the transmission of arboviruses. The increase of BRSV seroprevalence with

DEGURBA could be explained by the high exposure of European bison in smaller herds in comparison to larger ones (Table 2).

Table 4. Association between seroprevalences to bovine viral diarrhoea virus (BDVD), bovine herpesvirus type 1 (BoHV-1) and 4 (BoHV-4), bovine rhinovirus 3 (BRV3), bovine adenovirus type 3 (BAdV-3), and bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV), and Schmallenberg virus (SBV) in the free-living European bison populations ^{1–6} to human population densities and degree of urbanization at the district level.

Human Population Density (Person/km ²)	Seroprevalence (%)							
	BVDV	BoHV-1	BoHV-4	BRV3	BAdV-3	BRSV	BTV	SBV
19 ¹ –25 ²	0.5	1.1	15.4	46.3	64.7	19.1	44.1	70.3
31 ³ –32 ⁴	2.8	0	3.6	89.8	85.7	4.1	39.4	84.5
50 ⁵ –51 ⁶	0	0	3.5	50.9	75.5	24.5	23.7	59.4
χ^2	3.9	1.6	9.2	28.5	8.3	8.3	9.5	11.1
p ⁷	0.1	0.4	0.01	<0.001	0.02	0.02	0.009	0.004
Degree of Urbanization— DEGURBA (%)								
22 ³ –38.5 ⁶	0	0	12.5	60.0	90.0	10.0	44.4	72.2
41.7 ⁴ –42.9 ¹	2.2	0	4.5	63.2	72.1	5.9	32.2	81.0
50.5 ² –57.6 ⁵	0.4	0.9	11.4	53.1	70.0	22.5	40.4	66.8
χ^2	2.4	1.0	2.7	2.0	1.9	9.6	2.1	6.1
p ⁷	0.3	0.6	0.3	0.3	0.4	0.008	0.3	0.05

¹ Bieszczady (bieszczadzki district); ² the Białowieża Forest (hajnowski district); ³ Bieszczady (leski district); ⁴ the Knyszyńska Forest (sokólski district); ⁵ Borecka (giżycki district); ⁶ Knyszyńska (białostocki district); ⁷ significant differences marked in bold ($p < 0.05$).

3.4. Risk Factors

On the basis of multivariable analysis, several models were developed (Table 5). The increasing risk of virus exposure with age was the main finding for most virus models, and this can be explained by the commonly accepted principle that the longer an animal lives, the more likely it is to come into contact with a pathogen. The sanitary status was also found to be a risk factor for BAdV-3 and BTV seropositivity. However, in the case of BAdV-3 infection, the higher risk of infected animals among the eliminated European bison would be related to their health deterioration. In the case of BTV, which most likely stopped circulating in the country a few years earlier, this is probably connected with the older age of eliminated European bison and the long persistence of BTV antibodies. This is also consistent with the observed decreasing risk of BTV infection from year to year (Table 5). The frequency-dependence of SBV transmission was illustrated in the risk occurring in peaks in 2017 and 2022.

Table 5. Mixed effect generalized linear mixed models (GLMMs) for the analysis of risk factors of bovine rhinovirus 3 (BRV3), bovine adenovirus type 3 (BAdV-3), and bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV) and Schmallenberg virus (SBV) seropositivity in European bison.

BRV3 (N = 345)						
Variable	Category	Odds ratio (OR)	β (SE)	z	$p > z $	95%CI
Age group	≤1 year old	reference				
	2–3 years old	2.1	1.3	1.2	0.2	0.6–7.2
	≥4 years old	9.6	5.3	4.1	<0.001	3.2–28.3
Random effects		Variance	SEM	95% CI		
	origin	3.9	2.1	1.3–11.5		

Table 5. *Cont.*

BTV (N = 560)						
Variable	Category	Odds ratio (OR)	β (SE)	z	$p > z $	95%CI
Year	2016	reference				
	2017	0.4	0.2	−1.8	0.08	0.1–1.1
	2018	0.3	0.1	−2.5	0.01	0.1–0.8
	2019	1.3	0.7	0.4	0.7	0.4–3.9
	2020	0.2	0.1	−2.4	0.02	0.07–0.8
	2021	0.3	0.2	−2.3	0.02	0.09–0.8
	2022	0.2	0.1	−2.3	0.02	0.07–0.8
Age group	≤1 year old	reference				
	2–3 years old	0.4	0.3	−1.3	0.2	0.1–1.6
	≥4 years old	6.4	3.3	3.6	<0.001	2.3–17.5
Sanitary status	immobilized (healthy)	reference				
	eliminated	2.4	0.8	2.5	0.01	1.2–4.6
	fallen	2.0	0.9	1.5	0.1	0.8–4.9
	dead in traffic accident	1.1	0.9	0.1	0.9	0.2–5.4
Random effects	origin	Variance	SEM	95% CI		
		4.4	2.7	1.3–14.4		
SBV (N = 552)						
Variable	Category	Odds ratio (OR)	β (SE)	z	$p > z $	95%CI
Year	2016	reference				
	2017	3.1	1.5	2.4	0.02	1.2–7.9
	2018	1.5	0.6	1.0	0.3	0.7–3.3
	2019	0.5	0.2	−1.4	0.1	0.2–1.2
	2020	0.8	0.4	−0.5	0.6	0.3–2.0
	2021	0.6	0.2	−1.3	0.2	0.2–1.3
	2022	3.3	1.7	2.3	0.02	1.2–8.8
Age group	≤1 year old	reference				
	2–3 years old	3.2	1.1	3.7	<0.001	1.7–6.1
	≥4 years old	8.0	2.5	6.7	<0.001	4.3–14.7
Random effects	origin	Variance	SEM	95% CI		
		0.2	0.2	0.02–1.9		

4. Discussion

Our study has given insights on the endemic and emerging virus transmission threatening European bison, whose wild population is still increasing. While dynamic development of large herds becomes a success for restitution of the species, it creates dangers, which may hinder all efforts. Due to the increasing number of European bison, many populations roam more frequently close to farmlands, having more direct contacts with cattle and other farm animals in general, which may possibly increase the risk of disease transmission by expansion of the interface between the sylvatic and synanthropic environments. Additionally, anthropogenic factors such as human activity, population density, intensive farming, degree of urbanization, or touristic pressure may indirectly affect wildlife health

by impacting their use of space and movements, induce reproductive distress, exposure to environmental pollution, stress, and immunosuppression by further increasing susceptibility to infection [14]. Moreover, illegal but increasingly popular European bison baiting, tracking and approaching for photography may contribute to the risk of exposure and transmission of infectious agents from the areas inhabited by human and livestock.

We have not been able to demonstrate an increased risk of endemic infections of BoHV-1 and BVDV in European bison, which are highly prevalent in Polish cattle [15–17]. The transmission is most often by direct contact; however, indirect pathways through contaminated substances such as fomites present in the manure used as a fertilizer is also likely. As observed in a previous study initiated over a decade ago [7], the exposure to pestivirus (BVDV) and alphaherpesvirus (BoHV-1) is accidental and occurs in large free-living populations and mostly in free-roaming European bison bulls. Despite such a significant increase in the population size in recent years (Table S1), the prevalence and consequently the risk of infection with these viruses has not increased. However, these infections probably affect the European bison condition, as all the seropositive animals were selected by a vet supervising the population for culling due to their poor health conditions. Although transmission of these pathogens is relatively rapid in cattle, spread in European bison from the infected bulls does not occur. As for BVDV, it can be explained that transiently infected (TI) animals do not easily transmit the virus to other herd members in comparison to persistently infected (PI) in utero animals, which are the main source of infection. Herpesvirus infections are usually latent, where the virus persists in the lymphatic system throughout the lifespan, and it is spread transiently via secretions from the respiratory or genital tract. Therefore, the low seroprevalence of BoHV-1 which could be explained by the decreased species' sensitivity to infection should be considered as one explanation. Additionally, European bison may be exposed to other BoHV-1-like viruses from cervids, such as CvHV-1, which are also prevalent in Polish deer [18], but there is no evidence of this either. Some other explanations could be low prevalence of BVDV and BoHV-1 in neighbouring cattle herds; however, this is not supported by epidemiological data [17]. Yet another explanation could be the high standard of biosecurity measures that have been introduced on holdings due to the spread of African swine fever in the region [19].

The situation was slightly different in regard to BoHV-4 exposure in European bison which we have observed in the species for the first time. A previous study carried out in the late 1990s did not confirm BoHV-4 infections in European bison [20]. The virus is highly prevalent in dairy cattle and may cause disorders of female reproductive tract and fertility problems in cows. However, virus infections alone do not lead to diseases, as antibodies are also detected in healthy animals. It is likely to be the initiating factor for metritis and vulvovaginitis as it gives the entry for opportunistic bacteria such as *Escherichia coli*, *Trueperella pyogenes*, *Streptococcus* spp., and *Histophilus somni* [21]. Interestingly, the highest BoHV-4 seroprevalence was found in European bison from the Białowieska Forest, where an endemic disease called necrotic balanopostitis (NBP) has been one of the major health threats affecting 6–7% of bulls for over 40 years. BoHV-4 exposure was also confirmed in European bison from the Borecka and Knyszyńska Forests, which were created from the animals brought from the Białowieska Forest. Some cases of NBP were reported in the Borecka Forest [4] (pp. 297–302). Surprisingly, the only captive herd, where BoHV-4 antibodies were detected, was the Pszczyna population, where NBP cases were historically [4] (p. 297) and recently [22] observed. Despite that Borchers et al. [20] have not been able to confirm an association between NBP and BoHV-4 infection, our study provides new insights. Whether this would be a breakthrough in NBP research depends on the results of further studies. We have observed that the infection was density-dependent, and higher seroprevalence in free-living populations suggested a transmission from cattle. The antibodies to BoHV-4 in the tested European bison were present only in the adult (≥ 4 years) individuals, which are assumed to participate in the reproduction; therefore, this suggests sexual transmission. However, the cases of NBP were observed even in male calves in the Białowieska Forest [4]

(p. 299) [6]. Certainly, more research is needed to isolate BoHV-4 circulating in European bison and to characterize it. BoHV-4 is a representative of gammaherpesviruses, genetically and antigenically similar to malignant catarrhal fever virus (MCFV) found in African antelopes—Alcelaphine Herpesvirus-1 (AIHV-1) [23]. MCFV causes high mortality in most bovids, including European and American bison (*Bison bison*). Moreover, a specific to American bison gammaherpesvirus species named Bison lymphotropic herpesvirus (Bison-LHV) was identified [24]. The knowledge on BoHV-4 prevalence in wildlife is limited. A high BoHV-4 seroprevalence, approx. 70%, was observed in African buffalo [25], which probably has a cross-protective effect against lethal AIHV-1 infection. BoHV-4 circulating among cervids and mouflon in Hungary was confirmed to be homologous to bovine strains [26], which suggested a transmission from domestic cattle.

The seroprevalence of respiratory viruses was comparable with the results of the analysis conducted between 2011 and 2015 [7]. The data suggest that the viruses circulate in the population of European bison and/or are transmitted from cattle. Exposure to BRV3 was density-dependent; therefore, an increase of exposure may be expected if the European bison population size will continue to increase. Unlike in cattle, where these infections occur in calves and are associated with the development of Bovine Respiratory Disease (BRD), the risk of seropositivity in European bison increased with age. The connection between high seroprevalence of respiratory viruses and pulmonary pathological changes has already been described [6,7]. In our study, the exposure to those viruses was more frequent in European bison that were fallen or culled due to poor health conditions. We have also connected this exposure to cases of pasteurellosis, which have increased in the recent years [27].

In addition to the direct impact of anthropopressure on the studied environments, the impact of climate change and emergence of exotic to Europe pathogens as the effect of global changes was studied. We compared the cross-sectional and longitudinal distribution of infections with two emerging viruses transmitted through the same insect vector—*Culicoides* spp. midges. Infections with BTV and SBV have emerged in European bison in Poland simultaneously in 2012 [8,28]. While SBV has spread all over the country, BTV infections are limited almost exclusively in European bison populations closer to the eastern border of the country. Our study showed that BTV has not spread much further in subsequent years [8]. Earlier, BTV-14 was identified as the circulating strain, and, fortunately, the virus did not show much pathogenicity for European bison or other domestic ruminants. The source of the infection was identified as attenuated BTV-14, which might have contaminated some vaccines used illegally in eastern Europe [29]. Since then, BTV infections in domestic ruminants surveyed according to the BTV national monitoring program were more and more sporadic, and no new infections have been identified in the last few years (unpublished data), thus confirming the situation observed in European bison in this study, suggesting limited or absent circulation of the virus.

By contrast, SBV is most likely circulating in the environment with the cyclical waves of increasing seroprevalence in European bison, overlapping peaks of infection rates observed in domestic ruminants and insect vector in the country [30,31]. The habitat of vast forests inhabited by Polish European bison provides excellent conditions for insect breeding, while increasing winter temperatures prolong their activity, which extends the animal exposure to vector-borne diseases. Previously, we have observed that the number of *Culicoides* biting midges caught in the trap located in the Białowieska Forest outnumbered, by several dozen times, the numbers of those insects collected in the neighbouring cattle farm [32]. Additionally, the higher exposure rates of European bison in comparison to domestic ruminants and cervids were connected also with the size of the largest herbivore in Europe and thus with the amount of gas that is excreted, which is an attractant to insects. A similar association was observed for tick-borne encephalitis virus (TBEV) exposure in European bison with very high seroprevalences to the virus in the endemic areas in comparison to indicator cervid species [33]. As in the case of TBEV, BTV and SBV transmit in the frequency-dependent mode [9], which is less dependent on animal density and are

related more to environmental factors of the habitat and the availability of a competent vector, whose natural activity is seasonal or dependent on presence of other reservoirs [34]. An example of frequency-dependent exposure can also be demonstrated by the increase of *Thelazia* spp. invasion extensity in European bison from the Bieszczady Mountains in the south-eastern part of the country [35] compared to a similar-sized population in the Białowieska Forest, where fewer cases of thelaziosis were identified in the last few years [36].

The presented analysis constitutes a part of the conservation strategy and measures enshrined therein to protect the endangered species of the largest herbivore—European bison [37].

5. Conclusions

The findings of our epidemiological analysis may be concluded as follows:

- No significant increase in the interspecies and intraspecies risk of endemic viral infections was found in European bison in Poland in the last seven years;
- The free-living and larger European bison populations are more frequently exposed to some viral pathogens which impact their health;
- A new direction of research should include Bovine herpesvirus type 4 (BoHV-4) that may be relevant to the aetiology of the necrotic balanoposthitis in European bison bulls;
- No effect of anthropogenic factors on the transmission rates and frequency of selected viral infections was observed;
- Vector-borne bluetongue virus (BTV) is probably no longer circulating in the environment, while infection with emerging Schmallenberg virus (SBV) has become endemic with waves of seroprevalence increasing every 3–4 years;
- Veterinary supervision and passive monitoring of European bison herds is crucial as it leads to the identification and elimination of infected individuals and prevents further transmission;
- Active surveillance should be continued in order to monitor threats and to establish a further conservation strategy for the species.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/d15040535/s1>. Figure S1: The size of Polish European bison free-living populations by year. Figure S2: The size of Polish European bison captive populations by year. Table S1: The size of European bison populations included in the study by year. Table S2: List of ELISAs used in the serosurveillance of European bison exposure to bovine viral diarrhoea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), bovine rhinovirus 3 (BRV3), bovine adenovirus (BAdV-3), bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV), and Schmallenberg virus (SBV). Table S3: Primers and probes used for bovine adenovirus (BAdVs) and bovine rhinovirus 3 (BRV3). Table S4: Frequency and antibody levels in the sera of European bison in relation to bovine rhinovirus 3 (BRV3), bovine adenovirus type 3 (BAdV3), and bovine respiratory syncytial virus (BRSV) exposure.

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References

- Raczyński, J. *European Bison Pedigree Book 2016–2021*; Białowieża National Park: Białowieża, Poland, 2017–2022. Available online: https://bpn.com.pl/index.php?option=com_content&task=view&id=1133&Itemid=213 (accessed on 3 February 2023).
- The International Union for Conservation of Nature (IUCN) Red List of Threatened Species 2020. Available online: <https://www.iucnredlist.org/species/2814/45156279> (accessed on 3 February 2023).
- Wróblewski, K. *Żubr Puszczy Białowieskiej*; ZOO Garden Poznań: Poznań, Poland, 1927.
- Krasińska, M.; Krasiński, Z.A. *Żubr*, 2nd ed.; Drukarnia im. Półtawskiego: Kielce, Poland, 2017; 448p.
- Larska, M.; Krzysiak, M.K. Infectious diseases monitoring as an element of Bison *bonasus* species protection. In *Compendium of the European Bison (Bison bonasus) Health Protection*; Larska, M., Krzysiak, M.K., Eds.; National Veterinary Research Institute: Puławy, Poland, 2022; pp. 71–96.
- Krzysiak, M.K.; Dackiewicz, J.; Kęsik-Maliszewska, J.; Larska, M. Post-mortem evaluation of pathological lesions in European bison (*Bison bonasus*) in the Białowieża Primeval Forest between 2008 and 2013. *J. Vet. Res.* **2014**, *58*, 421–431. [[CrossRef](#)]
- Krzysiak, M.K.; Jabłoński, A.; Iwaniak, W.; Krajewska, M.; Kęsik-Maliszewska, J.; Larska, M. Seroprevalence and risk factors for selected respiratory and reproductive tract pathogen exposure in European bison (*Bison bonasus*) in Poland. *Vet. Microb.* **2018**, *215*, 57–65. [[CrossRef](#)] [[PubMed](#)]
- Krzysiak, M.K.; Iwaniak, W.; Kęsik-Maliszewska, J.; Olech, W.; Larska, M. Serological study of exposure to selected arthropod-borne pathogens in European bison (*Bison bonasus*) in Poland. *Transbound. Emerg. Dis.* **2017**, *64*, 1411–1423. [[CrossRef](#)] [[PubMed](#)]
- McCallum, H.; Barlow, N.; Hone, J. How should pathogen transmission be modelled? *Trends Ecol. Evol.* **2001**, *16*, 295–300. [[CrossRef](#)] [[PubMed](#)]
- Smith, M.J.; Telfer, S.; Kallio, E.R.; Burthe, S.; Cook, A.R.; Lambin, X.; Begon, M. Host-pathogen time series data in wildlife support a transmission function between density and frequency dependence. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 7905–7909. [[CrossRef](#)]
- Dohoo, I.; Martin, W.; Stryhn, H. *Veterinary Epidemiologic Research*, 2nd ed.; VER Inc.: Charlottetown, PE, Canada, 2010; 865p.
- Humphry, R.W.; Cameron, A.; Gunn, G.J. A practical approach to calculate sample size for herd prevalence surveys. *Prev. Vet. Med.* **2004**, *65*, 173–188. [[CrossRef](#)]
- Polska w Liczbach. Available online: <https://www.polskawliczbach.pl/> (accessed on 3 February 2023).
- Brearley, G.; Rhodes, J.; Bradley, A.; Baxter, G.; Seabrook, L.; Lunney, D.; Liu, Y.; McAlpine, C. Wildlife disease prevalence in human-modified landscapes. *Biol. Rev. Camb. Philos. Soc.* **2013**, *88*, 427–442. [[CrossRef](#)]
- Rola, J.G.; Larska, M.; Grzeszok, M.; Rola, J. Association between antibody status to bovine herpesvirus 1 and quality of milk in dairy herds in Poland. *J. Dairy Sci.* **2015**, *98*, 781–789. [[CrossRef](#)]
- Rypuła, K.; Płoneczka-Janeczko, K.; Czopowicz, M.; Klimowicz-Bodys, M.D.; Shabunin, S.; Siegwalt, G. Occurrence of BVDV infection and the presence of potential risk factors in dairy cattle herds in Poland. *Animals* **2020**, *10*, 230. [[CrossRef](#)]
- Rypuła, K.; Płoneczka-Janeczko, K.; Kita, J.; Kumala, A.; Żmudziński, J.F. Seroprevalence of BHV-1 (bovine herpesvirus type 1) among non-vaccinated dairy cattle herds with respiratory disorders. *Pol. J. Vet. Sci.* **2012**, *15*, 561–563. [[CrossRef](#)]
- Rola, J.; Larska, M.; Socha, W.; Rola, J.G.; Materniak, M.; Urban-Chmiel, R.; Thiry, E.; Żmudziński, J.F. Seroprevalence of bovine herpesvirus 1 related alphaherpesvirus infections in free-living and captive cervids in Poland. *Vet. Microbiol.* **2017**, *204*, 77–83. [[CrossRef](#)] [[PubMed](#)]
- Frant, M.P.; Gal-Cisoń, A.; Bocian, Ł.; Ziętek-Barszcz, A.; Niemczuk, K.; Woźniakowski, G.; Szczotka-Bochniarz, A. African swine fever in wild boar (Poland 2020): Passive and active surveillance analysis and further perspectives. *Pathogens* **2021**, *10*, 1219. [[CrossRef](#)] [[PubMed](#)]

20. Borchers, K.; Brackmann, J.; Wolf, O.; Rudolph, M.; Glatzel, P.; Krasinska, M.; Krasinski, Z.A.; Frölich, K. Virologic investigations of free-living European bison (*Bison bonasus*) from the Bialowieza Primeval Forest, Poland. *J. Wildl. Dis.* **2002**, *38*, 533–538. [[CrossRef](#)]
21. Wathes, D.C.; Oguejiofor, C.F.; Thomas, C.; Cheng, Z. Importance of viral disease in dairy cow fertility. *Engineering* **2020**, *6*, 26–33. [[CrossRef](#)] [[PubMed](#)]
22. Hławiczka, M.; Zuber, Ł.; Bielecki, W. An attempt to cure necrotic foreskin inflammation (NZN) posthitis in a European bison. In *Żubry w Puszczy Augustowskiej*; SMŻ: Augustów, Poland; pp. 45–48. Available online: <https://smz.waw.pl/wp-content/uploads/2021/04/Conference-Materials.pdf> (accessed on 7 February 2023).
23. Thiry, E.; Dubuisson, J.; Bublot, M.; Van Bresseem, M.F.; Pastoret, P.P. The biology of bovine herpesvirus-4 infection of cattle. *DTW* **1990**, *97*, 72–77.
24. Li, H.; Gailbreath, K.; Flach, E.J.; Taus, N.S.; Cooley, J.; Keller, J.; Russell, G.C.; Knowles, D.P.; Haig, D.M.; Oaks, J.L.; et al. A novel subgroup of rhadinoviruses in ruminants. *J. Gen. Virol.* **2005**, *86*, 3021–3026. [[CrossRef](#)] [[PubMed](#)]
25. Dewals, B.; Gillet, L.; Gerdes, T.; Taracha, E.L.; Thiry, E.; Vanderplasschen, A. Antibodies against bovine herpesvirus 4 are highly prevalent in wild African buffaloes throughout eastern and southern Africa. *Vet. Microb.* **2005**, *110*, 209–220. [[CrossRef](#)]
26. Kálmán, D.; Egyed, L. PCR detection of bovine herpesviruses from nonbovine ruminants in Hungary. *J. Wildl. Dis.* **2005**, *41*, 482–488. [[CrossRef](#)]
27. Kedrak-Jablońska, A.; Budniak, S.; Szczawińska, A.; Reksa, M.; Krupa, M.; Krzysiak, M.; Szulowski, K. Isolation and identification of *Pasteurella multocida* from European bison in Poland. *Post. Mikrobiol. Supl.* **2017**, *56*, 69–70.
28. Larska, M.; Krzysiak, M.; Smreczak, M.; Polak, M.P.; Zmudziński, J.F. First detection of Schmallenberg virus in elk (*Alces alces*) indicating infection of wildlife in Białowieża National Park in Poland. *Vet. J.* **2013**, *198*, 279–281. [[CrossRef](#)]
29. Koltsov, A.; Tsybanov, S.; Gogin, A.; Kolbasov, D.; Koltsova, G. Identification and characterization of bluetongue virus serotype 14 in Russia. *Front. Vet. Sci.* **2020**, *7*, 26. [[CrossRef](#)]
30. Kęsik-Maliszewska, J.; Collins, Á.B.; Rola, J.; Blanco-Penedo, I.; Larska, M. Schmallenberg virus in Poland endemic or re-emerging? A six-year serosurvey. *Transb. Emerg. Dis.* **2021**, *68*, 2188–2198. [[CrossRef](#)] [[PubMed](#)]
31. Larska, M. Schmallenberg virus: A cyclical problem. *Vet. Rec.* **2018**, *183*, 688–689. [[CrossRef](#)] [[PubMed](#)]
32. Kęsik-Maliszewska, J.; Krzysiak, M.K.; Grochowska, M.; Lechowski, L.; Chase, C.; Larska, M. Epidemiology of Schmallenberg virus in European bison (*Bison bonasus*) in Poland. *J. Wildl. Dis.* **2018**, *54*, 272–282. [[CrossRef](#)] [[PubMed](#)]
33. Krzysiak, M.K.; Anusz, K.; Konieczny, A.; Rola, J.; Salat, J.; Strakova, P.; Olech, W.; Larska, M. The European bison (*Bison bonasus*) as an indicatory species for the circulation of tick-borne encephalitis virus (TBEV) in natural foci in Poland. *Ticks Tick-Borne Dis.* **2021**, *12*, 101799. [[CrossRef](#)]
34. Cuéllar, A.C.; Kjær, L.J.; Kirkeby, C.; Skovgard, H.; Nielsen, S.A.; Stockmarr, A.; Andersson, G.; Lindstrom, A.; Chirico, J.; Lühken, R.; et al. Spatial and temporal variation in the abundance of *Culicoides* biting midges (Diptera: Ceratopogonidae) in nine European countries. *Parasites Vectors* **2018**, *11*, 112. [[CrossRef](#)]
35. Filip-Hutsch, K.; Laskowski, Z.; Myczka, A.W.; Czopowicz, M.; Moskwa, B.; Demiaszkiewicz, A.W. The occurrence and molecular identification of *Thelazia* spp. in European bison (*Bison bonasus*) in the Bieszczady Mountains. *Sci. Rep.* **2022**, *12*, 22508. [[CrossRef](#)]
36. Demiaszkiewicz, A.W.; Moskwa, B.; Gralak, A.; Laskowski, Z.; Myczka, A.W.; Kołodziej-Sobocińska, M.; Kaczor, S.; Plis-Kuprianowicz, E.; Krzysiak, M.; Filip-Hutsch, K. The nematodes *Thelazia gulosa* Raillet and Henry, 1910 and *Thelazia skrjabini* Erschov, 1928 as a cause of blindness in European bison (*Bison bonasus*) in Poland. *Acta Parasitol.* **2020**, *65*, 963–968. [[CrossRef](#)]
37. Olech, W.; Perzanowski, K. (Eds.) *European Bison (Bison bonasus) Strategic Species Status Review 2020*; IUCN SSC Bison Specialist Group and European Bison Conservation Center: Warsaw, Poland, 2022; pp. 1–138. Available online: <http://ebcc.wisent.org/science-papers/> (accessed on 3 February 2023).

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