

Table S1. The size of European bison populations included in the study by year.

Origin	population type	2015	2016	2017	2018	2019	2020	2021	increase 2015-2021 (individuals)	% increase 2015- 2021	mean % increase/year
Białowieża Forest	free-ranging	578	596	654	519	770	715	779	201	34.8	5.1
Borecka Forest	free-ranging	107	108	120	112	112	117	125	18	16.8	2.6
Knyszyńska Forest	free-ranging	134	144	158	158	184	214	212	78	58.2	7.9
Bieszczady	free-ranging	344	402	487	551	668	707	729	385	111.9	13.3
Zachodniopomorskie herds	free-ranging	184	205	216	265	305	334	340	156	84.8	10.8
Augustowska Forest	free-ranging	0	0	0	8	9	17	20	12	150.0	35.7
Pszczyna (Jankowice)	captive	43	50	42	48	56	45	50	7	16.3	2.5
Białowieża reserves	captive	28	39	43	19	27	31	28	0	0.0	0.0
Niepołomice	captive	27	26	27	23	22	19	16	-11	-40.7	-8.4
Muczne (Bieszczady)	captive	12	16	11	11	9	13	10	-2	-16.7	-3.0
Jabłonowo	captive	6	9	8	9	10	7	9	3	50.0	7.0
ZOO Warszawa	captive	6	5	7	6	8	7	9	3	50.0	7.0
Miedzyzdroje	captive	7	8	9	9	6	8	8	1	14.3	2.3
Bałtów	captive	8	9	8	8	8	6	7	-1	-12.5	-2.2
Gołuchów	captive	11	12	10	7	5	6	7	-4	-36.4	-7.3
ZOO Gdańsk	captive	10	12	13	12	13	5	7	-3	-30.0	-5.8
Zoo Poznań	captive	2	2	3	5	5	6	7	5	250.0	23.2
Pszczyna Park	captive	8	7	7	6	7	5	6	-2	-25.0	-4.7
ZOO Bydgoszcz	captive	3	3	3	4	4	3	4	1	33.3	4.9
Szewce	captive	0	0	0	0	0	5	3	3	n.a	n.a
Ustroń	captive	4	4	5	3	3	3	2	-2	-50.0	-10.9
Kiermusy	captive	6	7	8	8	6	4	0	-6	n.a	n.a.
Smardzewice	captive	7	6	6	0	0	0	0	-7	n.a	n.a.
ZOO Łódź	captive	3	1	0	0	0	0	0	-3	n.a	n.a.
total		1538	1671	1845	1791	2237	2277	2378	832	54.1	7.5
	free-ranging	1347	1455	1635	1613	2048	2104	2205	858	63.7	8.6
	captive	191	216	210	178	189	173	173	-18	-9.4	-1.6

*not applicable

Figure S1. The size of Polish European bison free living populations by year.

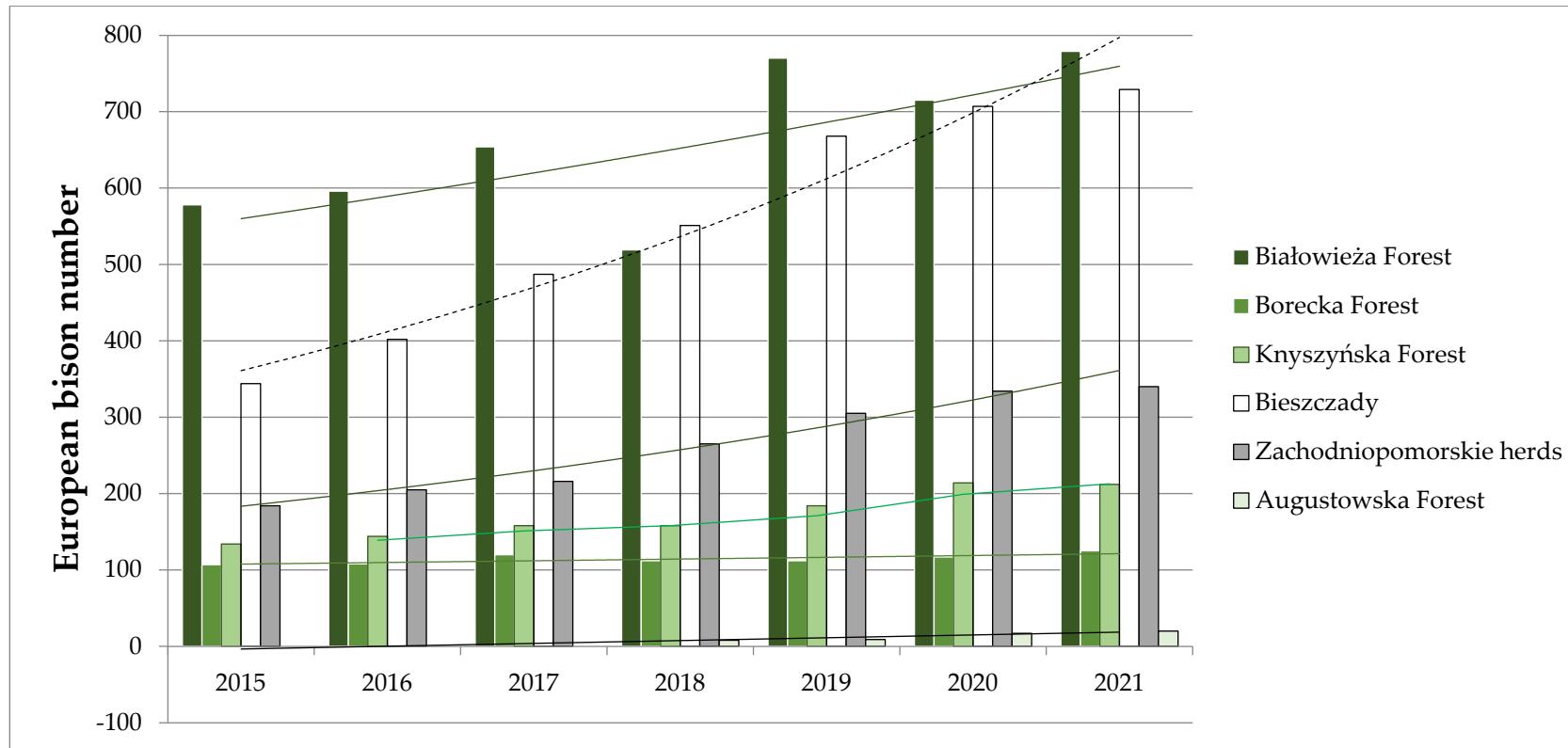


Figure S2. The size of Polish European bison captive populations by year.

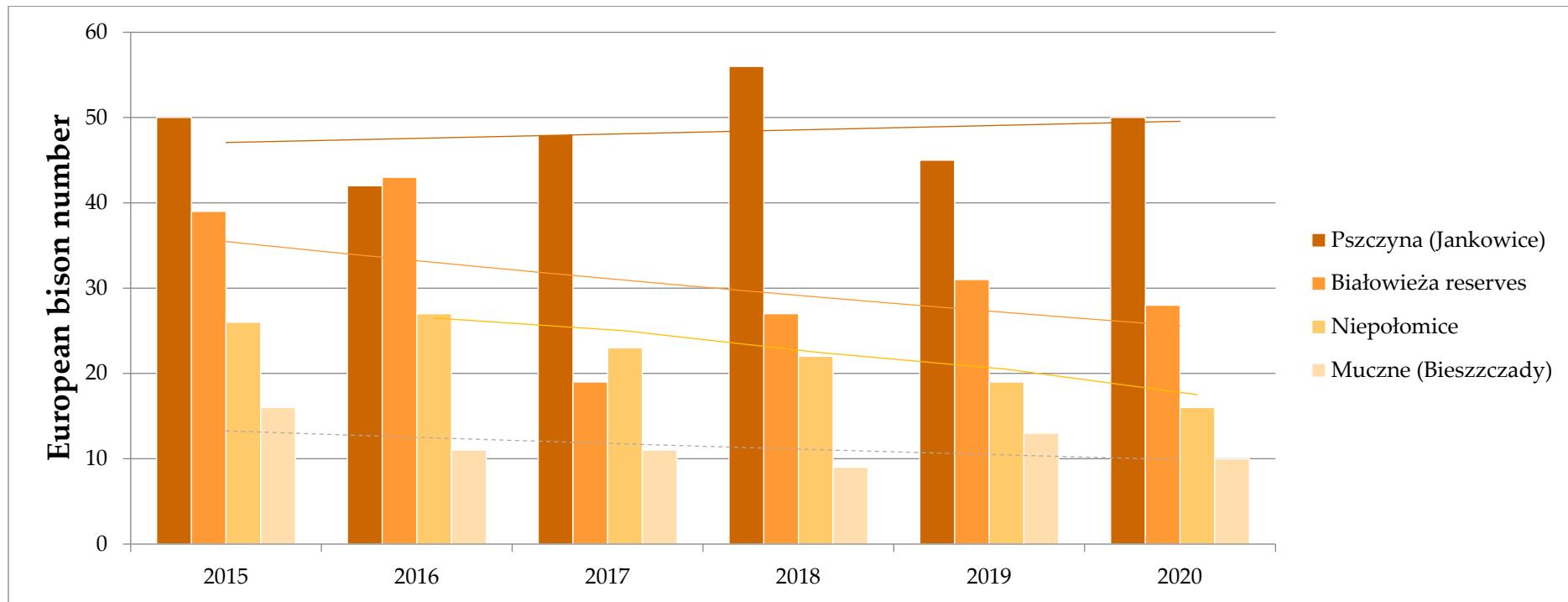


Table S2. List of ELISAs used in the serosurveillance of European bison exposure to bovine viral diarrhea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), bovine rhinovirus 3 (BRV3), bovine adenovirus (BAdV-3), bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV) and Schmallenberg virus (SBV).

Virus	Kit	Cut-off	Sensitivity	Specificity	Reference
BVDV	Indirect IDEXX BVDV Ab Test (IDEXX Laboratories, Inc., Liebefeld-Bern, Switzerland)	S/P≥0.3	96.3%	95.0%	manufacturer
BoHV-1	IBR gB X3 Antibody Test kit and (IDEXX Laboratories, Inc., Liebefeld-Bern, Switzerland)	S/N≥55%	99.8%	100%	manufacturer
BoHV-4	Monoscreen AbELISA BoHV-4 (Bio-X Diagnostics S.A., Rochefort, Belgium)	S/P>30%			
BRV3	Trivalent Antibody Test kit (IDEXX Montpellier SAS, Montpellier, France)***	S/P≥20%*	98.8%	86.7%	manufacturer
BAdV-3			75.0%	100%	
BRSV			not provided	not provided	
BTV	Ingezim BTV DR (Ingenaza, Madrid, Spain)	0.15× \bar{x} O.D. positive control	98.5%	99.5%	[Niedbalski, 2011]
SBV	ID Screen ELISA Schmallenberg virus Competition Multi-species (ID.vet, Grabels, France)	S/N≤40%	100%	97.6%	[Pejaković et al., 2018]

*The results was also graduated accordingly to S/P values between weak positive (+) to very strong positive (++++)

Niedbalski, W. Evaluation of commercial ELISA kits for the detection of antibodies against bluetongue virus. *Pol J Vet Sci.* 2011, 14, 615-619. <https://doi.org/10.2478/v10181-011-0091-y>

Pejaković, S.; Wiggers, L.; Coupeau, D.; Kirschvink, N.; Mason, J.; Muylkens, B. Test selection for antibody detection according to the seroprevalence level of Schmallenberg virus in sheep. *PLoS One* 2018, 13, e0196532. <https://doi.org/10.1371/journal.pone.0196532>

Table S3. Primers and probes used for bovine adenovirus (BAdVs) and bovine rhinovirus 3 (BRV3)

Target	Primer/probe	Sequence (5'-3')	Amplicon size (bp)	Gene/protein	Reference
BAdV	BAdV-Hex-33F	GAAATGCGAGGTATCTGTCT			
	BAdV-Hex-138R	TGWTGGAGCTACAAAAGGATCTCTAA		hexon protein gene	
	BAdV-HEX-65-TaqMan	TGCAGTTCATCACTGCCACWCAAAGC			
	BPI3F	TGTCTTCCACTAGATAGAGGGATAAAATT			
BRV3	BPI3R	GCAATGATAACAATGCCATGGA		protein M gene	69 [Horwood et al. 2010]
	BPI3P-FAM-TAMRA	ACAGCAATTGGATCAATAA			

Hakhverdyan, M. Development of a TaqMan RealTime PCR Assay for the Rapid Detection of Bovine Adenovirus Serotypes in Cattle. J Vet Med Res. 2016, 3,7.

Horwood, P. F.; Mahony, T. J. Multiplex real-time RT-PCR detection of three viruses associated with the bovine respiratory disease complex. J Virol Meth. 2011, 17, 360-363.
<https://doi.org/10.1016/j.jviromet.2010.11.020>.

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.

	BRV3		BAdV-3		BRSV	
	n	%	n	%	n	%
(weak positive) +	27	7.1	72	19.1	28	7.41
++	51	13.5	61	16.1	13	3.44
+++	58	15.3	39	10.3	11	2.91
++++	20	5.3	22	5.8	4	1.06
(very strong positive) +++++	8	2.1	31	8.2	4	1.06
negative	214	56.6	153	40.5	318	84.13
total	378	100	378	100	378	100