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Post-mortem evaluation of pathological lesions in European bison (*Bison bonasus*) in the Białowieża Primeval Forest between 2008 and 2013

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Abstract

The study presents the analysis of the findings of 234 post-mortem examinations on free-ranging and captive European bison selectively culled or having fallen between 2008 and 2013 in Białowieża Primeval Forest. Pneumonia, emphysema, nephritis, bodily traumas, and intestinal lesions were observed in 106 (45.3%), 77 (32.9%), 82 (35.0%), 68 (29.1%), and 56 (23.9%) animals respectively and were the most common pathological changes. Almost half of all males (66 out of 140; 47.1%) tested showed some pathological changes of prepuce and penis, described as posthitis or balanoposthitis. Infection with liver fluke (*Fasciola hepatica*) and lungworm (*Dictyocaulus viviparus*) was observed macroscopically in 114 (48.7%) and 80 (32.9%) bison respectively. *F. hepatica* prevalence was associated with the emergence of other liver changes such as hepatitis and cirrhosis ($P < 0.001$). Similarly, the prevalence of *D. viviparus* coincided with pneumonia ($P = 0.001$), changes in the upper respiratory tract ($P = 0.04$), and emphysema ($P < 0.001$). Hepatitis, infection with *F. hepatica*, and pathological lesions in the male and female reproductive tracts were associated with the animals' age. Mechanical injuries, caused by other bison or less commonly by traffic accidents, were the most common cause of death of bison below six months of age. Most pathological changes were significantly more frequent in the selectively culled animals in comparison with the ones having fallen, which confirms the desirability of elimination as a tool to improve the health and welfare of the bison population and limit the number of reservoirs of invasive and possibly infectious diseases.

Keywords: European bison, pathological lesions, *Dictyocaulus viviparus*, *Fasciola hepatica*, Poland.

Introduction

The European bison *(Bison bonasus)* is the largest European mammal. The males may reach 1000 kg (average 700-750 kg) of weight and females 650 kg (average 400-450 kg). The last indigenous free-ranging European bison in the Białowieża Primeval Forest was killed just after the First World War, in 1919. After 10 years of absence, European bison were reintroduced to Białowieża Forest, and a committed restoration has been carried out since 1929 (5). Today's bison population was founded by twelve animals from the Lowland-Caucasian line, which had survived in zoos. Only seven of them started the Białowieża bison breeding line, therefore the inbred rate is high (30). On 31 December 2012, the size of the European bison population was estimated at 4978 in the world, 1074 in Poland, and 504 in the Białowieża Forest (34). Despite the success of the restoration, the European bison is still considered endangered and any health threats should be carefully monitored. Infections and parasite invasions are the main risks for the European bison population. The species is often exposed to bovine pathogens, which can easily cross the interspecies barrier (39). Foot-and-mouth disease and blackleg may cause high morbidity and mortality of European bison; however their significance is rather historical since they either have been eradicated or are rare in domestic ruminants in the country (21, 39). Since 1996, when bovine tuberculosis was first detected in European bison (20), lesions caused by *Mycobacterium tuberculosis* in fallen and eliminated animals have been observed in the population of the Bieszczady Mountains (20). Currently, the main health issue for

European bison males in Białowieża Primeval Forest is enzootic disease causing necrotic-purulent changes in the prepuce and/or penis, defined as posthitis or balanoposthitis (21). In severe and chronic cases, the injuries may even lead to autoamputation of the penis. Despite the significant involvement of many researchers, the source of balanoposthitis has not been discovered (16, 24, 38). Newly emerging diseases in cattle also raise the risk for European bison by cross-species infection. In 2013, antibodies specific to bluetongue virus (BTV) were detected in the bison from Białowieża Primeval Forest, which was followed by the identification of BTV-14 (http://www.defra.gov.uk/animal-diseases/files/poa-btv14-20121130.pdf; Larska *et al.*, in prep.). The transmission of a new *Orthobunyavirus* called Schmallenberg virus (SBV) to the population of Białowieża European bison in 2012 has also been described (23). New threats notwithstanding, since the early beginnings of European bison introduction, have invariably been exposed to parasitic invasions, especially liver fluke (*Fasciola hepatica*) and lungworm *Dictyocaulus* sp. (8, 12, 21, 39). The parasites infecting the bison from Białowieża Forest have been described in detail (10); however, the dynamics of parasite fauna in bison is quite rapid resulting in the emergence of new species such as the recently identified blood-sucking nematode *Ashworthius sidemi* (29). In the area of Białowieża, parasitological surveys continue constantly. One of the methods for sizing the parasitic infection problem is the parasitological post-mortem examination of culled animals (9).

The aim of this study was to evaluate the health status of the European bison population in Białowieża during the last five years, including the extensiveness of infection with liver fluke and lungworm.

Material and Methods

A total of 234 European bison (140 males and 94 females) were necropsied at the Białowieża National Park (BNP) between September 2008 and December 2013. In addition to general visual inspection, the internal organs such as the liver, lungs, heart, and kidneys were sectioned. The liver was cut open along the lobes from the porta to expose the bile ducts. Additionally, liver crosscuts were performed if necessary. The lungs were sectioned starting from the trachea and continuing along from the main to smaller bronchi. Then the lungs were sliced starting from the diaphragmatic lobes. The heart was cut open along the long axis to visualise the two atria and ventricles. The kidneys were sectioned on halves along the axis starting from the cortex into the renal pelvis.

The age of the animals varied from a few weeks to 26 years. The bison found dead accounted for 47.9% (112 individuals), while the selectively culled comprised 52.1 (122 individuals) of the investigated animals. Most animals were free-ranging bison (212 individuals; 90.6%), while the rest (22 individuals; 9.4%) were captive bison from the BNP fenced reserve areas. The animals were divided into three age groups: calves (one year of age or below), young bison (two to three years old) and adults (four years old or above) according to the division implemented during the winter inventories at BNP (21). Adult bison are those that have reached sexual maturity. The data on the population size and structure used in the statistical analysis derived from the archives of the BNP (34). Selective culling was performed according to the decision of the Minister for the Environment at the BNP or the General Director for Environmental Protection outside the BNP. The elimination decisions derogate from the ban on the killing of protected animals contained in the Act of 16 April 2004 on Nature Conservation (Journal of Laws of 2004, No. 92, item 880). According to the BNP Conservation Plan, the health status of the Białowieża bison population is subject to monitoring, which implies the need to perform a post-mortem inspection of each fallen or eliminated bison. The data analysed post-mortem included identification of the animal (sex, registration or pedigree no., age, approximate weight, place and circumstances of death), pathological changes, and endoparasites observed macroscopically~~,~~. Data sets derived from the veterinary protocols prepared at each necropsy were stored in the BNP archives. For the selectively culled bison, those protocols also included the criterion for the elimination.

STATA version 11 software (StataCorp., USA) was used in the statistical analysis and graph design. The results were expressed as the mean values with 95% confidence interval (CI) calculated for the binomial distribution using the Wald estimator. The association between the presence/absence of individual pathological lesions or parasite infection and binomial predictors such as fallen *versus* culled and free-ranging *versus* captive was analysed using the Mann-Whitney test. Defining the relations between the proportion of animals with specific lesions or parasitic infection and nominal or categorical variables such as month, year, and age group was achieved by the Kruskal-Wallis rank sum test. The age of the animals was represented by two variables: age in years (discrete) and age group (ordinal). Significant differences ($P < 0.05$) were described in the value of χ^2 and/or the probability value (P). The multivariate logistic regression model (logit) was used to analyse the relationships between all variables and regression coefficients (coef.), and P values were generated.

Results

Occurrence of pathological lesions. In all 234 bison subjected to post-mortem examination, pneumonia, emphysema, and nephritis were the most

common pathological changes in 106 (45.3%), 77 (32.9%), and 82 (35.0%) animals respectively (Table 1). Body traumas and lesions of the intestines were similarly common and occurred in 68 (29.1%) and 56 (23.9%) bison respectively. Almost half of all males (66 out of 140; 47.1%) showed some pathological changes of the prepuce and penis described as posthitis or balanoposthitis (Fig. 1.). Hepatic steatosis (3%), eye cataracts (4.3%), and renal polycystitis (7.7%) were very rarely occurring lesions (Table 1).

Cryptorchidism, epididymal cysts, and testicular atrophy were present in 31 (22.1%) males (data not included in tables/figs). Prolapse of the penis was observed in one case. The frequency of these changes was comparable in the males with posthitis/ balanoposthitis (16/66; 24.2%) and in the rest of the males (15/74; 20.3%).

In the age groups, the lowest frequency of the changes in the reproductive tract was in the youngest bison (14.3%), while the highest occurrence was in the eldest animals (25.3%); however, the difference was not statistically significant. Most pathological changes were independent of the age group ($P > 0.05$), except for the inflammatory changes in the liver ($\chi^2=11.2$; $P = 0.004$), changes in the prepuce and penis ($\chi^2=10.8$; $P = 0.005$), and lesions in the reproductive tract of females ($\chi^2=7.4$; $P = 0.02$) (Table 1).

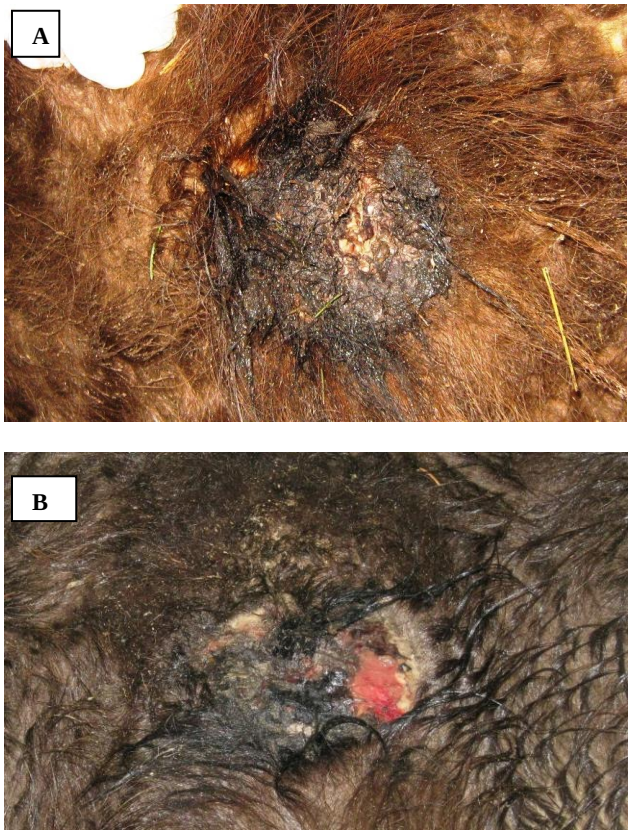


Fig. 1. Two cases of necrotic balanoposthitis in European bison males with glans penis autoamputation and visible ulcerative lesions (B)

Prevalence of *Fasciola hepatica* and *Dictyocaulus viviparus*. Macroscopic examination of the endoparasites found in the carcasses revealed frequent infection with *F. hepatica*. The 114 (48.7%) animals evinced this trematode (Table 1). The proportion of animals infected with *F. hepatica* was related to the age group ($\chi^2=11.6$; $P = 0.003$), with the lowest percentage among the youngest bison ≤ one year of age (30.5%) and the highest among the animals > four-year-old (57.1%). The association between *F. hepatica* presence in the liver and the animal age was significant using logistic regression ($P = 0.045$) (Fig. 2). The frequency of fluke detection was also related to the month of the examination ($P = 0.004$) (data not shown). In addition, *F. hepatica* prevalence was associated with the incidence of other changes such as hepatitis, cirrhosis ($P < 0.001$), and to a lesser degree steatosis *(*$P = 0.04$) as tested by the multiple logistic regression model.

The adult *D. viviparus* organisms were found in the trachea and/or bronchi of 80 (34.2%) animals. No association between animal age or the month of study and lungworm presence was observed; however, the prevalence of the parasite coincided with pneumonia ($P = 0.001$), changes in the upper respiratory tract ($P = 0.04$), and emphysema ($P < 0.001$).

Differences between the lesions in European bison found dead and selectively culled. The summary of descriptive statistics is given in Table 2. Out of 234 animals necropsied, 112 (47.9%, SE 3.3, 95% CI 41.4-54.3%) were fallen bison. The age groups were represented equally. The percentages of animals found dead rather than culled ≤ one-year-old, two to three years old, and ≥ four years old were 50.8% (SE 6.5, 95%CI 37.5-64.1%), 47.7% (SE 7.7, 95%CI 32.0-63.6%), and 46.6% (SE4.3, 95%CI 37.9-55.6%). The lesions were significantly more frequent in the selectively culled bison than in the animals found dead, except for hepatic steatosis, which occurred more frequently in the latter group (Table 2). A significantly higher occurrence ($\chi^2 = 25.3$; $P = 0.0001$) of lesions of the reproductive tract other than prepuce/penis was observed in the selectively culled males (35.7%; SE 5.4; 95%CI 26.9-48.1%) than in the fallen males (16.7%, SE 1.6; 95%CI 0-4.9). No association between the cause of death and the occurrence of lesions in the upper respiratory tract, heart, eyes, or traumatic lesions was found.

According to the multivariable logistic regression model, the presence of *D. viviparus* (coef. = 1.3; $P = 0.02$) and *F. hepatica* (coef. = 1.8; $P < 0.001$) infections, hepatic cirrhosis (coef. = 1.8; $P = 0.015$), hepatitis (coef .= 2.1; $P = 0.001$), nephritis (coef . = 3.5; $P < 0.001$), lesions of the prepuce and penis (coef. = 1.5; $P < 0.001$), and other lesions in males (coef. = 3.6; $P = 0.001$) positively correlated with selective elimination of animals. While analysing the data, it was considered that in 38 (42.6%) of fallen bison, a full necropsy could not been performed due to the

advanced decomposition of the carcass or its fragmentation by scavengers.

Differences between the lesions in free-ranging and captive European bison. No significant differences in most of the pathological changes or endoparasitic infections between these two groups were observed when tested using the Mann-Whitney test and the logistic regression model (P > 0.05) (data not presented). Notwithstanding this, the percentage (51.6%) of prepuce and penis lesions in the free-ranging bison was significantly (P = 0.01) higher than in captive males (7.1%). Conversely, lesions of the reproductive tract in females were significantly (P = 0.0008) more frequent in captive (37.5%) than in free-ranging bison cows (11.6%).

Occurrence of pathological changes of the prepuce and penis in European bison males between 2008 and 2013. As presented above, the frequency of prepuce and penis lesions was related to the age group (χ^2 = 10.8; P = 0.005) (Table 1). Similarly, these lesions were associated with age when tested the by logit test (coef. = -0.6; P = 0.03) (Fig. 3). The highest percentage of prepuce/penis lesions, reaching approx. 60%, was observed in the males between two and seven years of age (Fig. 3).

The percentage of bison males with prepuce and penis lesions in particular years is presented in Table 3. A decrease in this percentage was found in 2012 and 2013; however, it was not statistically significant (P > 0.05). The frequency of prepuce and penis lesions was not associated with the month of detection (data not shown).

The percentage of the male bison study population so affected is presented graphically in Fig. 4. The mean percentage of males with an observed case was 5.3%, with the maximum value of 9.0% in 2010 and minimum value of 1% in 2008. The mean percentage of fallen males (1.3%) was significantly lower (χ^2 = 20.5; P = 0.0001) than in culled animals (4.0%).

Post-mortem investigations of causes of mortality in calves six months of age or below. Forty eight (20.5%) out of the 234 European bison included in the study were at or below six months of age. Of those 48, 26 were calves which had been found dead and they constituted 54.2% of all calves ≤ half a year old; 14.4% of all fallen animals; and 11.1% of all bison investigated. No statistical differences between the frequency of lesions observed in the youngest fallen bison and the frequency in the older (> six months of age) animals were found (Table 4). A significantly higher detection rate of *D. viviparus* (P = 0.05) and upper respiratory tract lesions (P = 0.02) characterised calves ≤ six months of age in comparison with older bison. Conversely, *F. hepatica* infection (P = 0.01) and lesions in the female reproductive tract (P = 0.03) were significantly more frequent in older animals than in calves ≤ six months of age. The most common cause of death of the youngest bison (42.3%) was mechanical injury caused by other bison or less commonly by traffic accents.

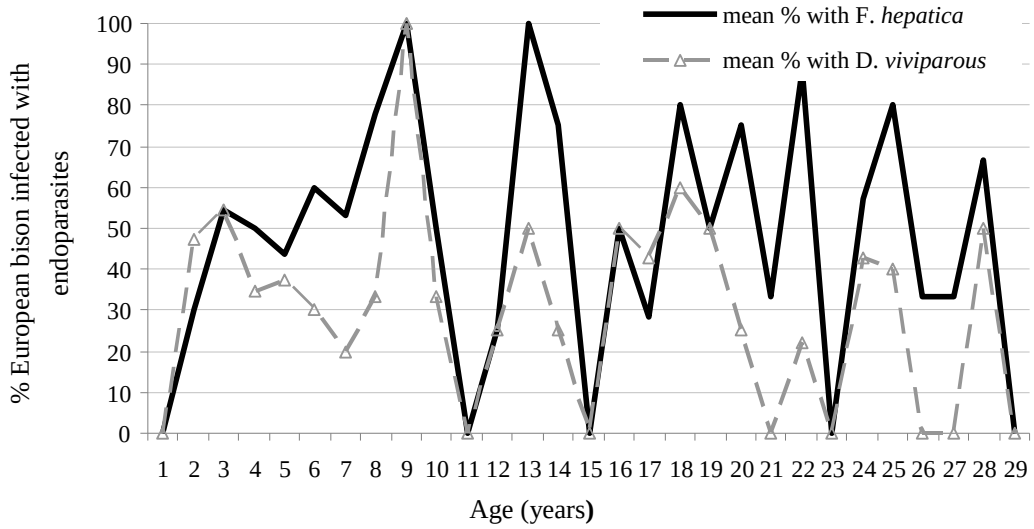


Fig. 2. Percentage of *Dictyocaulus viviparus*- (black line) and *Fasciola hepatica*- (dashed line) positive European bison by animal age

Table 1. Pathological changes and endoparasites (*Dictyocaulus viviparus* and *Fasciola hepatica*) observed during post-mortem examination of European bison and arrayed by age (group I – calves up to one year of age; group II – young animals between two and three years of age; group III – adult animals at or over four years of age). The number (n) and proportion (%) of animals showing lesions/endoparasite infection, standard error (SE), and 95% confidence interval (CI) calculated using the Wald estimator are presented. The asterisks indicate those lesion/parasite presences, which differed significantly ($P \leq 0.05$) between the age groups according to the Kruskal-Wallis equality of populations rank test. [1] and [2] refer to the number of males (N = 35; 27; 78) and females (N = 24; 15; 55) in each age group respectively

Bison	Parameter	*D. viviparus*	Lungs		Upper respiratory tract	*F. hepatica**	Liver			Intestine	Kidneys		Heart	Prepuce / penis[1]*	Reproductive tract of females[2]*	Eye	Trauma	No lesions
			emphysema	inflammation			cirrhosis	steatosis	inflammation*		inflammation	polycystitis				cataract		
Group I (N = 59)	n	25	16	23	9	18	7	3	6	12	23	3	5	9	0	0	18	11
	%	42.4	27.1	39.0	15.3	30.5	11.9	5.1	10.2	20.3	39.0	5.1	8.5	25.7	0	0	30.5	18.6
	SE	6.4	5.8	6.3	4.7	6.0	4.2	2.9	3.9	5.2	6.3	2.9	3.6	7.4	0	0	6.0	5.1
	95%CI	28.8–55	15.8–38.5	26.5–51.4	6.1–24.4	18.8–42.3	3.6–20.1	0–10.7	2.5–17.9	10.1–30.6	26.5–51.4	0–10.7	1.4–15.6	11.2–40.2	6.1	0	18.6–42.4	8.5–28.7
Group II (N = 42)	n	15	14	17	5	20	8	0	12	10	17	2	5	18	1	0	6	7
	%	35.7	33.3	40.5	11.9	47.6	19.0	0	28.6	23.8	40.4	4.8	11.9	66.7	6.7	0	14.3	16.7
	SE	7.4	7.3	7.6	5.0	7.7	6.1	0	7.0	6.6	7.6	3.3	5.0	9.1	6.4	0	5.4	5.7
	95% CI	21.5–52.0	19.6–49.5	25.6–56.7	39.8–25.6	32.0–63.6	8.6–34.1	8.4	15.7–44.6	12.0–39.4	25.6–56.7	0.6–16.2	4.0–25.6	46.0–83.5	0.2–31.9	0	5.4–28.5	7.0–31.3
Group III (N = 133)	n	40	47	66	13	76	23	4	28	34	42	13	16	39	12	10	44	21
	%	30.1	35.3	49.6	9.8	57.1	17.3	3.0	21.0	25.6	31.6	9.8	12.0	50.0	21.8	7.5	33.1	15.8
	SE	4.0	4.1	4.3	2.6	4.3	3.3	1.5	3.5	3.8	4.0	2.6	2.8	5.6	5.6	2.3	4.1	3.2
	95% CI	22.4–38.6	27.2–44.1	40.8–58.4	5.3–16.1	48.3–65.7	11.3–24.9	0.8–7.5	14.5–29.0	18.4–33.8	23.8–40.2	5.3–16.1	7.0–18.8	38.4–61.5	11.8–35.0	3.7–13.4	25.2–41.8	25.2–41.8
Total (N = 234)	n	80	77	106	27	114	28	7	46	56	82	18	26	66	13	10	68	39
	%	34.2	32.9	45.3	11.5	48.7	16.2	3.0	19.7	23.9	35.0	7.7	11.1	47.1	13.8	4.3	29.1	16.7
	SE	3.1	3.1	3.2	2.1	3.3	2.4	1.1	2.6	2.8	3.1	1.7	2.1	4.2	3.5	1.3	3.0	2.4
	95% CI	28.1–40.3	26.8–39	39–51.8	7.4–15.7	42.3–55.2	11.5–21	0.7–5.1	14.5–24.8	18.4–29.4	28.9–41.2	4.2–11.1	7–15.2	38.8–55.5	6.7–21	16.6–68.8	23.2–35	11.9–21.5

Table 2. Pathological changes and endoparasites (*Dictyocaulus viviparus* and *Fasciola hepatica*) observed during post-mortem examination of European bison and arrayed by manner of dying (found dead *versus* selectively culled). The number (n) and proportion (%) of animals showing lesions/endoparasite invasion, standard error (SE), and 95% confidence interval (CI) calculated using the Wald estimator are presented. The P-values generated for the association between two groups using the Mann-Whitney test are given at the bottom. [1] and [2] refer to the number of males (N = 60; 80) and females (N = 52; 42)) in each category respectively

Bison	Parameter	*D. viviparus*	Lungs		Upper respiratory tract	*F. hepatica*	Liver			Intestine	Kidneys		Heart	Prepuce / penis[1]	Reproductive tract of females[2]	Eye	Trauma	No lesions
			emphysema	inflammation			cirrhosis	steatosis	inflammation		inflammation	polycystitis				cataract		
Fallen (N=112)	n	10	19	31	9	18	6	6	6	16	6	2	13	16	0	3	35	39
	%	8.9	17.0	27.7	8.0	16.1	5.4	5.4	5.4	14.3	5.4	1.8	11.6	26.7	0	26.7	31.3	34.8
	SE	2.7	3.5	4.2	2.6	3.5	2.1	2.1	2.1	3.3	2.1	1.3	3.0	5.7	0	1.5	4.4	4.5
	95%CI	3.6–14.2	10–23.9	19.4–36	3 –13.1	9.3–22.9	1.2–9.5	1.2–9.5	1.2–9.5	7.8–20.8	1.2–9.5	0–4.2	5.7–17.5	15.4–37.8	0	–0.3–5.7	22.5–40	25.9–43.8
Culled (N=122)	n	70	58	75	18	96	32	1	40	40	76	16	13	50	13	7	33	0
	%	57.4	47.5	61.5	14.8	78.7	26.2	0.8	32.8	32.8	62.3	13.1	10.7	62.5	30.5	5.7	27.0	0
	SE	4.5	4.5	4.4	3.2	3.7	4.0	0.8	4.3	4.3	4.4	3.1	2.8	5.4	7.1	2.1	4.0	0
	95% CI	48.6–66.2	38.7–56.4	52.8–70.1	8.5–21	71.4–86	18.4–34	0–2.4	24.5–41.1	24.5–41.1	53.7–70.9	7.1–19.1	5.2–16.1	52–73.1	17–44.9	1.5–9.9	19–35	0
P value		<0.0001	<0.0001	<0.0001	0.1	<0.0001	<0.0001	0.04	<0.0001	0.0009	<0.0001	0.001	0.8	0.0001	0.0001	0.25	0.5	0.0001

Table 3. Occurrence of prepuce/penis lesions in fallen animals, selectively culled, and all European bison bulls tested arrayed by year of testing

European bison males	Parameter	Year					
		2008	2009	2010	2011	2012	2013
Fallen (N = 60)	n	0	1	8	5	0	2
	%	0	11.1	47.1	55.6	0	18.2
	SE	0	11.1	12.5	17.6	0	12.2
	95% CI	0	–10.9–33.1	22.4–71.7	20.8–90.3	0	–5.9–42.3
Culled (N = 80)	n	2	14	10	9	7	8
	%	100	70.0	66.7	26.3	53.8	57.1
	SE	0	10.5	12.6	12.8	14.4	13.7
	95% CI	0	49.2–90.8	41.7–91.6	30.9–81.6	25.4–82.3	30–84.3
Total (N = 140)	n	2	15	18	14	7	10
	%	50.0	51.7	56.3	56.0	28.0	40.0
	SE	28.9	9.4	8.9	10.1	9.2	10.0
	95% CI	–7.1–107.1	33–70.4	38.6–73.9	36–76	9.8–46.1	20.2–59.8

Table 4. Pathological changes and endoparasites (*Dictyocaulus viviparus* and *Fasciola hepatica*) observed during post-mortem examination of European bison calves below six months of age. The number (n), proportion (%) of animals showing lesion/endoparasite infection, standard error (SE), and 95% confidence interval (CI) calculated using the Wald estimator are presented. [1] and [2] refer to the number of males (N = 25; 16) and females (N = 23; 10) in each category respectively. The asterisks indicate the lesions/parasite presence observed in the calves ≤ six months of age, which differed significantly (P ≤ 0.05) from all the animals above this age (data not shown)

European bison calves	Parameter	*D. viviparus*	Lungs		Upper respiratory tract	*F. hepatica*	Liver			Intestine	Kidneys		Heart	Penis / prepuse[1]	Reproductive tract of females[2]	Eye	Trauma	Lesions absent
			emphysema	inflammation			cirrhosis	steatosis	inflammation		inflammation	polycystitis				cataract		
Fallen (N = 26)	n	2	4	4	1	2	0	2	0	1	2	0	2	4	0	0	11	8
	%	7.7	15.4	15.4	3.8	7.7	0	7.7	0	3.8	7.7	0	7.7	25.0	0	0	42.3	30.8
	SE	5.3	7.2	7.2	3.8	5.3	0	5.3	0	3.8	5.3	0	5.3	11.1	0	0	9.9	9.2
	95%CI	–3.3–18.7	0.5–30.2	0.5–30.2	–4.1–11.8	–3.3–18.7	0	–3.3–18.7	0	–4.1–11.8	–3.3–18.7	0	–3.3–18.7	19.2–48.1	0	0	22–62.7	11.8–49.8
Total (N = 48)	n	19	11	19	9	12	4	3	4	10	18	1	4	6	0	0	16	8
	%	39.6*	22.9	36.6	18.8*	25.0*	8.3	6.3	8.3	20.8	37.5	2.1	8.3	24.0	0*	0	33.3	16.7
	SE	7.1	6.1	7.1	5.7	6.3	4.0	3.5	4.0	5.6	7.1	2.1	4.0	8.7	0	0	6.9	5.4
	95% CI	25.2–53.9	10.6–35.2	25.2–53.9	7.3–30.2	12.3–37.7	0.2–16.4	–0.8–13.3	0.2–16.4	8.9–32.7	23.3–51.7	–2.1–6.3	0.2–16.4	6–42	0	0	19.5–47.1	5.7–27.6

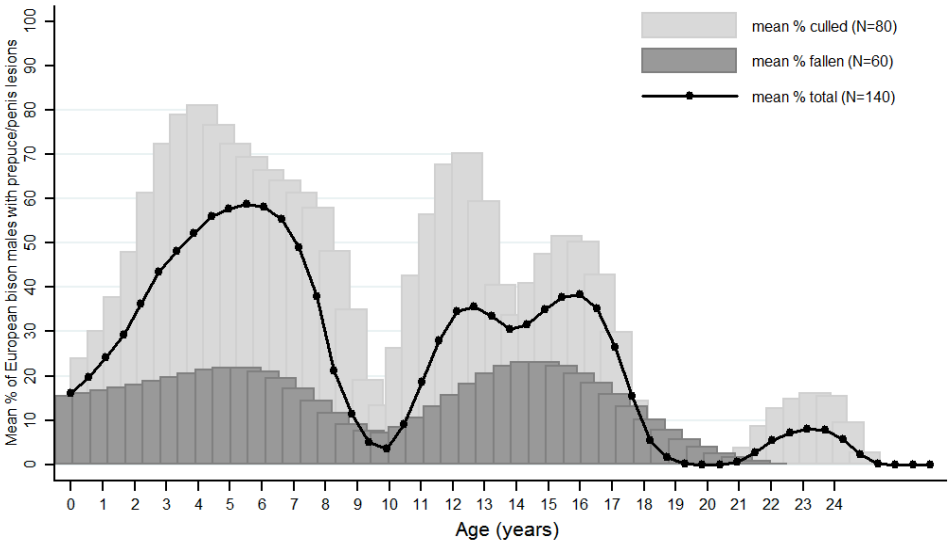


Fig. 3. Percentage of prepuce/penis lesions depending on European bison age presented as kernel-weighted local polynomial smoothed columns (light grey – selectively culled; dark grey - fallen), and curve (all males)

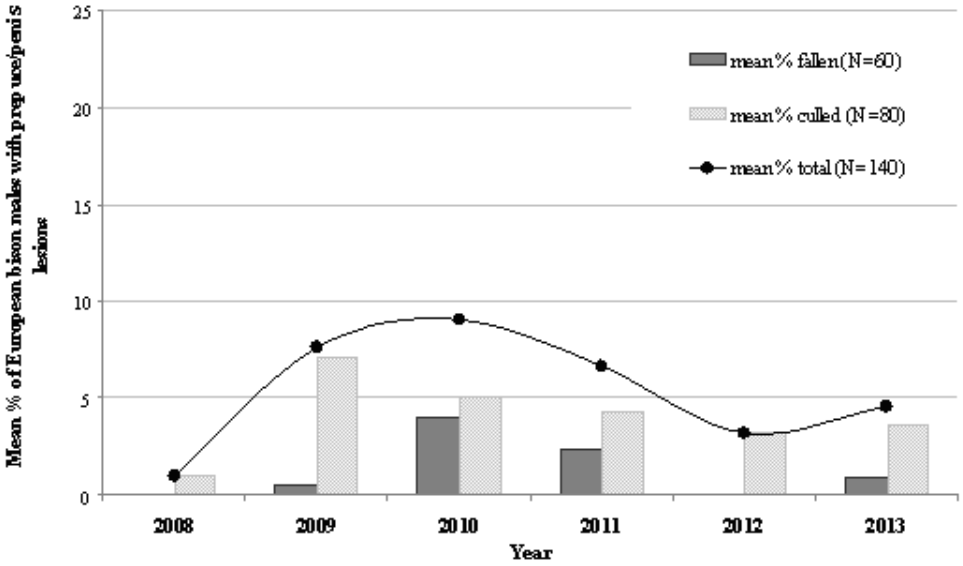


Fig. 4. The yearly proportion of prepuce/penis lesion-affected males as percentages of the male bison study population according to the European bison count Annual Reports (BNP Archive). The smoothed curve represents mean values for all males, while columns stand for fallen (dark grey) and selectively culled (light grey)

Discussion

Similarly to the observations of Piusiński *et al.* (31), pathological changes in the respiratory system were the most commonly observed lesions in the European bison population in Białowieża between 2008 and 2013. Catarrhal-purulent pneumonia caused by *Dictyocaulus viviparus* infection was the most frequent disease, as observed previously (8, 31, 39). Interstitial pneumonia, described previously in microscopic examination (31), was rarely detected in this study. In some cases, pneumonic lesions, coinciding with pleuritis with parietal and visceral pleura adhesions, were similar to lesions observed by Piusiński *et al.* (31).

The observed prevalence of *D. viviparus* was lower than that described in previous years (8, 10). This was probably related to the method used to evaluate the extensiveness of the lungworm invasion and also related to the inclusion of both eliminated and fallen bison in this study. Detecting *D. viviparus* larvae in faecal samples, Demiaszkiewicz *et al.* (8), found the nematode even in 100% of them; however, they tested only the eliminated bison. As our study presented, *D. viviparus* was significantly more frequent in the selectively culled bison than in fallen animals.

Dróżdż *et al*. (10), who investigated lungworms by postmortem examination of eliminated European bison in the 80's, described the prevalence of the lungworms at 61.5%, which is comparable with our results (54.7%). In fallen European bison, the infection with *D. viviparus* was observed only in 8.9% cases. The significant difference in the infection rate of *D. viviparus* between selectively culled and fallen animals seems to be important. It suggests that pulmonary nematodes may adversely affect the health of European bison, which are subsequently selected for elimination; however, the invasion itself may seldom cause animal death. As early as 1927, Wróblewski (39) noticed that the *Strongylidae* lungworms, which include *D. viviparus*, were often found in the carcasses of European bison which had died of pleuropneumonia caused by microorganisms such as *Pasteurella* sp. Demiaszkiewicz *et al.* (8) suggested greater extensiveness and intensiveness of the infection with *D. viviparus* in calves under the age of one year, which was explained by the immaturity of their immune system.

Similarly in the present study, the prevalence of *D. viviparus* (39.6%) and changes in the upper respiratory tract (18.8%) were significantly more frequent in the youngest calves below six months of age compared with the older European bison (Table 4). However, such differences were not observed if only fallen calves were considered, suggesting again that the infection with the lungworms does not lead directly to the death of the animal, but may affect the animal's welfare and by damaging the respiratory system engage secondary infections. The positive correlation between dictyocaulosis, pneumonia, and emphysema can be explained by the fact that the lesions may also occur secondarily to the infection with *D. viviparus* (31). However, when the prevalence of *D. viviparus* was compared in the three age groups and compared to age as a continuous variable, no association was found between animal age and infection rate. This correlation was observed for the second most common endoparasite in European bison - *Fasciola hepatica*. Identical findings were described by Gill (12). The observed *F. hepatica* infection in nearly 50% of the population of European bison is connected to the wide dispersion of this parasite in the Białowieża Primeval Forest (7). Almost a century ago, Wróblewski (39) described *F. hepatica* infection as the most frequent cause of mortality of European bison in Białowieża. Most authors indicate the relationship between the prevalence of *F. hepatica* and age of the European bison (7, 8, 31). The highest percentage of fluke infecting European bison was observed in the oldest animals, the lowest in individuals less than six months of age, which suggested that young animals have not been exposed to the parasite's intermediate host – the freshwater snail *Galba truncatula*. This could be related to late calving when low temperatures inhibit the life cycle of snails and their habitat, water reservoirs, are often frozen. The youngest calves are mainly suckling their mother's milk, which also limits the possibility of infection with *F. hepatica* during pasture. *F. hepatica* infection may lead to the formation of inflammatory foci in the liver or even to cirrhosis, also described previously (31). As in the case of *D. viviparus*, *F. hepatica* infection may increase the animal's susceptibility to other infections and emaciate the organism, however the infection itself probably does not cause the animal's death but rather becomes chronic. Therefore, the statistic for mortality caused by liver flukes, reaching even 62%, should be interpreted carefully, as other pathogens were not studied (9). In the present study, *F. hepatica* was almost five times more frequent in the selectively culled European bison (78.7%) than in the fallen animals (16.1%), suggesting the independence of animal mortality from infection with the fluke. Similarly, *F. hepatica* infection in cattle is rarely fatal but rather predisposes the host to secondary bacterial or viral infections. Additionally, *F. hepatica* and *D. viviparus* co-infection was observed in the present study, which may indicate a mutual increase in susceptibility. Experimental infection with *F. hepatica* of American bison (*Bison bison*), although subclinical, induced a decrease in haemoglobin and packed cell volumes, and an increase in eosinophils, which may significantly impact the performance of the immune system (11).

According to Kozak-Cięszczyk (19), the incidence of *F. hepatica* in cattle in central and eastern Poland was approximately 34.9%. Similarly, in water buffalos reared in Notecka Primeval Forest, the prevalence of *F. hepatica* was 32% (18). The mean extensiveness of liver fluke infection in the examined European bison was slightly higher than that described in cattle and water buffalos. This may be related to more frequent exposure of free-ranging animals to the intermediate host, as well as the impossibility of deworming the bison. Environmental factors such as higher water acidity can also affect the invasiveness of *F. hepatica* (27), which should be considered when planning the eradication/control strategy against these parasites in bison. In addition, Demiaszkiewicz (7) identified a relative of the *F. hepatica* fluke, *Parafasciolopsis fasciolaemorpha*, (25) present in 9% of European bison. The parasite is prevalent mainly in elk in Europe, but it may be also transmitted to other cervids and domestic ruminants (7, 25).

A higher frequency of pathological changes in selectively culled European bison compared with fallen animals reconfirmed the argument for the selection and elimination of animals. The bison selected are sick, weak, and often cachectic. The severity of parasitic infection and the pathological changes confirmed the need for culling to improve the welfare of the herd and entire population. Moreover, Anusz *et al.* (1) showed that along with the organ disorders observed at the necropsies in the eliminated European bison, some important biochemical and morphological blood

parameters such as leukocyte counts, concentrations of urea, creatinine, and alkaline phosphatase, and haematocrit are changed. An important element of the restoration breeding of endangered species should be the elimination of sick animals or those suspected of carrying disease, representing reservoirs of infection which pose an epizootic threat to other animals.

The carcasses of eliminated European bison are a very valuable source of information for veterinarians, but also for other scientists involved in bison research. It should be remembered that the observed differences between the fallen and eliminated animals are influenced by respectively worse and better access to the material. Free-ranging European bison may be found fallen after more than a few hours or even after a few months. The size of the animal usually helps the BNP caretakers or forest workers in finding the fallen animal. Such carcasses decompose quickly and then the material holds little value for the investigator (15). The European bison carcasses fall prey to scavengers, which usually start feeding on more exposed body areas such as the abdominal and thoracic cavity.

Steatosis was the lesion observed more frequently in fallen animals. Steatosis often occurs as a result of inappropriate nutrition of the captive European bison, therefore, it was diagnosed more often in animals examined directly after death. Intensive nutrition may lead to disorders like steatosis or fatty liver syndrome (26).

One of the greatest European bison health threats since it was first diagnosed in 1980, has been posthitis and balanoposthitis (3, 17). So far, the aetiology of the disorders has not been fully explained. The involvement of viral factors such alphaherpesvirus and pestiviruses has been excluded (3, 16). The lesions of the prepuce and penis vary in severity and in the most severe cases may lead to autoamputation of the penis. Bacteria such as *Corynebacterium* sp., *Bacillus* sp., *Fusobacterium* sp., and *Arcanobacterium* sp., and parasite protozoa from *Trypanosoma* genus have frequently been identified in the material collected from the lesions (14, 16, 17, 24). Microfilariae of *Onchocerca* sp. have been also suspected for being involved in balanoposthitis (1, 6). In this study, posthitis/balanoposthitis was mainly diagnosed in young animals (two to seven years of age) as described previously (4, 21). The lower frequency of posthitis/balanoposthitis in older bulls suggested that the elimination of males with the disease was effective. The presence of pathological changes of the prepuce and penis in younger males before reproductive age suggests that the infection is not transmitted sexually, as demonstrated by other authors (21). A higher incidence of the disease in the free-ranging male European bison population than in animals kept in captivity suggests the influence of some environmental factors, which has also been discussed elsewhere (4, 14, 17). Despite the selective culling conducted for almost 30 years, the incidence of posthitis/balanoposthitis in European bison in the Białowieża Primeval Forest indicates that action has not eliminated this problem at the population level. Between 1980 and 2002, Krasińska and Krasiński (21) estimated the annual occurrence of these lesions in Białowieża bison at 1.5%-14.8% (average 6.5%). In this study covering 2008 to 2013, the lesions were found in 1%-9% (average 5.3%) of males (Fig. 4).

Another problem discussed in this paper was the attempt to analyse the pathological changes in the youngest bison in relation to their mortality. The most common findings in fallen calves were the upper respiratory tract lesions and the presence of *D. viviparus*, but still they were not the predominant ones among the causes of mortality in this age group. As described previously (16, 39), a large proportion of fallen calves included in this study showed mortal traumas. The injuries in European bison are often caused by other individuals (39) and affect both juveniles and adults, *e.g.* bulls fighting over a female in oestrus (16). The injuries caused by human activity are less frequent (2). Increased mortality of calves born late in the year may also be due to the fact that these animals do not have an adequate layer of body fat, and are less resistant to severe weather conditions (37). Based on a similar intensity in young and older bison of pathological lesions often associated with the invasion of endoparasites, Gill (12) suggests that European bison calves are characterised by particularly low innate immunity. The mortality rate in young animals in the free-ranging population is difficult to define, as finding the carcasses in the field on time is rarely possible. Natural mortality of European bison in the Primeval Forest is quite low, ranging between 2.8% and 3.9% and not affecting the population size significantly (35). The analysis of the entire population dynamics of the Carpathian bison including the Białowieża population showed comparable survival rates of calves (0.93), young bison (0.97), and adults (0.98) (32). In the studied period, the fallen calves at or below six months of age represented 23.2% (26/112) of all fallen European bison. Thus, European bison calf mortality can be estimated at approx. 1%. Therefore, the innate immunity of European bison calves, suggested as being particularly low, (12) is probably actually comparable to or even more efficient than the immunity of bovine calves to which protective vaccinations and deworming programmes apply. No data on the mortality of bovine calves in Poland is available. In two countries of more intensive cattle farming, France and Norway, where biosecurity measures and infectious disease control programmes are monitored, the average mortality rate reaches 6.7% and 7.8% of calves respectively (13, 33). The main causes of bovine calf mortality include diarrhoea and respiratory system infections by viral and bacterial agents rarely identified in European bison (3, 35). However, their low seroprevalence also means that the European bison population remains naive to many pathogens common in cattle. Therefore, the risk of

transmission of the infection from livestock or other wildlife is considerable and so the health status of European bison should be constantly monitored. The outbreak of foot-and-mouth disease with a high European bison mortality, or the tuberculosis difficult to eradicate currently afflicting the herd in the Bieszczady Mountains support this opinion (20, 39).

In conclusion, the importance of the postmortem examination for monitoring a wildlife population's health status and planning activities for the improvement of its welfare should be emphasised (28). Routine necropsies secure valuable material for other research purposes and some emerging diseases threatening animal and human health can be identified expeditiously (20, 23). Surveillance over the health status of free-living animals, particularly the endangered species like European bison, is of great importance both for nature conservation in Poland, as well as for the domestic economy, since controlling infectious diseases in wildlife may help to prevent the transmission of pathogens to livestock (36).

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