

Introduced European bison (*Bison bonasus*) in a confined forest district: A ten year parasitological survey

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ABSTRACT

In the year 2012 a total of seven individuals (one bull and six cows) of European bison (*Bison bonasus*) were introduced to a fenced 200 ha area in a forest district (Almindingen, Island Bornholm, Denmark) in the Baltic Sea. In 2017 the herd was supplemented by introduction of another bull. The animals all originated from a population in the Polish Bialowieza forest. Faecal samples were recovered with intervals and subjected to a copro-parasitological investigation (applying egg flotation, sedimentation and Baermann technique). In addition, parasites were sampled during necropsy of animals euthanized or found dead three years after introduction. The animals carried a range of parasite types including trematodes (liver fluke *Fasciola* and rumen fluke), cestodes (*Moniezia*), nematodes (*Dictyocaulus viviparus*, trichurids, and other gastrointestinal nematodes (GIN) including *Trichostrongylus axei* and *Haemonchus contortus*). Egg and larval morphology also suggested presence of GIN genera *Nematodirus*, *Ostertagia* and *Cooperia*. The trematodes did not establish a life cycle in the recipient area, as judged by examination of calves born in the new area, but the cestode and several nematode species occurred in these animals. Especially *Dictyocaulus viviparus* was successfully spread and occurred together with GIN at moderate to high infection intensities at most sampling points. The reproduction among bison in the herd was high during the first few years. The exact birth rate is unknown but up to 24 calves are believed to be born during the ten year study period. Mortality among adults and calves occurred and in early June 2022 the total number of live animals was 11 (seven males, three females, one calf). The possible impact of the parasite infections on the bison health and future wildlife infection status is discussed.

1. Introduction

The European bison (*Bison bonasus*) is considered an endangered species. It was eradicated as a wild population during or shortly after the first World war (1914–18), whereafter a species restoration project was established based on a few surviving animals in captivity (reviewed by Krasinska and Krasinski, 2007). The main region of distribution is at present the Polish and Belarussian parts of the Bialowieza forest, but these large herbivores also occur in Russia, Ukraine and Slovakia and have been transferred to other regions in Europe including Denmark. In May 2012 the Danish Nature Agency initiated a five year stocking project on the Danish island of Bornholm in the Baltic Sea. A total of seven individuals (one bull and six cows) were captured in the Polish Bialowieza forest and relocated to the forest Almindingen in the centre of the island of Bornholm, Denmark. The animals were kept fenced in a 200 ha area of mixed deciduous-coniferous forest. The purpose was to manipulate the vegetation by introducing these large herbivores in order

to elevate biodiversity (Didriksen, 2015; Hartvig et al., 2021; Naturstyrelsen, 2022). The European bison flocks in their original area of distribution, the Bialowieza forest, have been subjected to a wide series of parasitological investigations revealing numerous protozoan and metazoan parasitic infections in the hosts. These were listed by Krasinska and Krasinski (2007), but recent studies have updated and completed data (Bien et al., 2010; Karbowski et al., 2014a,b; Demiaszkiewicz et al., 2018). The introduction of these large herbivores, which may carry a range of unique parasites, to new areas, without previous history of this host species, raises some questions. First of all, the introduction of alien parasite species with the introduced host to an ecosystem may have health consequences for endemic species already present in the area (Weinstein and Lafferty, 2015). Secondly, any parasite-induced effect on the European bison itself (Osinska et al., 2010), may challenge the health and welfare of the species in its new environment (Kołodziej-Sobocinska et al., 2018). In addition, these activities can raise ethical and public concern challenging social

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acceptance (Gamborg et al., 2010). In all cases, parasitological investigations of any herd will elucidate its health status, welfare level and give an overview of the interactions between environment and introduced animals. This information may be useful for management and propagation of the endangered species in question. Due to the complicated life cycle of some helminths a parasitological survey may also provide valuable ecological information about the recipient area. Presence of certain parasites in a specific area will indicate presence of the intermediate host organisms due to the fact that some trematodes use gastropods as obligate intermediate hosts and some cestodes involve arthropods in their life cycle. We have therefore performed an investigation of the introduced bison over ten years, from 2013 and until spring 2022. The study was based on sampling of freshly delivered dung (faeces) from the grazing areas taken with intervals and subjected to coprological investigation (flotation, sedimentation and Baermann isolation) and in some cases larval cultivation. During necropsy of animals found dead, conducted by the local veterinarian, we recovered adult nematode and cestode parasites. Parasites obtained from necropsies and larval cultures were subjected to morphometric and molecular diagnosis and supplemented the diagnoses based on the direct faecal examinations.

2. Materials and methods

2.1. Study area

The forest Almindingen is a state-owned forest and the fenced area comprising 200 ha (coordinates 55.1031–55.1208° N, 14.9317–14.96355° E) is located centrally on the island of Bornholm, Denmark, in the Baltic Sea. The tree vegetation in the fenced recipient area is mainly comprised of Norway spruce *Picea abies*, spruce forest clearings mainly populated by birch (*Betula* spp.), and smaller areas of oak (*Quercus* spp.) and beech (*Fagus sylvatica*). Extensive layers of bryophytes cover the forest ground. A smaller lake (Svinemose) is located centrally in the fenced area. The shore zone is populated by various species within the grass and half grass families. Common genera are *Carex*, *Juncus*, *Typha* and *Phragmites*. The herbs selected by the grazing bison following introduction are listed by Hartvig et al. (2021). Roe deer (*Capreolus capreolus*) from a large endemic population on the island has access to the fenced area through sluice openings placed with intervals along the lower part of the fence. Two male specimens of fallow deer (*Dama dama*) were present in the enclosure.

2.2. Animals

In the year 2012 (May 23) a total of seven specimens (1 bull and seven cows) of European bison (*Bison bonasus*) were transported from Poland (captured in the Bialowieza forest) and released in the fenced area within the following days. Within the first two years 6 calves were born (Didriksen, 2015). In 2017 an additional bull from the same region

of origin was introduced and one bull was exported to another animal resort in Denmark. In 2018 it was estimated that a total of 20 calves had been born since 2012 (Husted, 2018). No newborn calves were registered in 2019 and 2020, in 2021 three calves were born and in 2022 one calf (Table 1). On this basis around 31 *Bison bonasus* would be expected to occur in the study area. At the end of this study period 11 specimens (six males, four females, one calf) were confirmed alive (Table 1).

2.3. Faecal sampling

Faecal (dung) sampling was conducted after observing the flocks at a distance of 100 m. It was attempted to note where specific animals were delivering faeces at specific points. When the animals had moved from the site, we went into the grazing area and recovered freshly delivered and warm (near body temperature) faecal samples from the ground. Samples were sampled and kept in clean plastic bags or beakers and cooled to 3–5 °C within 30 min after recovery, until they were processed for examination within 24–48 h. Samplings were conducted in 2013 (three times), 2015, 2017, 2018, 2019, 2020, 2021 and 2022 at different time points during the seasons. The number of samples recovered varied from 3 to 19 per year.

2.4. Recovery of intact adult parasites

In three cases animals were found dead in the enclosure and the local veterinarian conducted a standard necropsy. Easily detectable parasites recovered from bronchi and intestines during necropsy were conserved in 70% ethanol and brought to the laboratory for further morphological and molecular diagnosis.

2.5. Egg and larval counts and measurements

Recording of eggs and larvae was performed by flotation (modified concentration McMaster), sedimentation, Baermann isolation and larval cultures (Monrad et al., 1999) (Fig. 1). On two occasions (2013 and 2015) gastrointestinal nematode (GIN) larvae (Fig. 2) were hatched from eggs, and the total length and the length of tail sheath extension (µm) were measured of 25–30 larvae/sample, aiming for a tentative diagnosis (genus or species) according to Bowman (1999) and Wyk et al. (2004). In 2022 individual larvae from larval cultures were isolated and subjected to molecular analysis.

2.6. Morphological diagnosis

Nematodes isolated from bronchi of the examined bison and conserved in ethanol were mounted on microscope slides applying mounting medium Aquatex (Merck, Germany) and covered with cover slips (Fig. 3). A part of a cestode (without scolex) isolated from the intestine was cut into two pieces. One part was used for molecular identification and the other part subjected to histology. It was dehydrated in

Table 1

Introduced and locally born European bison (*Bison bonasus*) in the 200 ha fenced forest area (Almindingen, Island of Bornholm, Baltic Sea).

Year	Introduced males	Introduced females	Calves born in the study site (minimum)	Confirmed mortality	Faecal sampling from herd	Necropsy of dead animals	Export of animals
2012	1	6	0	0			
2013			1 (male)	0	19		
2014			5 (3 males, 2 females)	0			
2015			4	2 cows, 1 calf	12	1 cow	
2016			1 (female)	1 moribund bull euthanized		1 old bull	
2017	1		2	2 male calves	4	2 male calves	1 bull
2018			2	1 female calf, 1 bull	8	1 female calf, 1 bull	
2019			0	0	4		
2020			0	0	3	0	
2021			3 (1 female, 2 males)	1 female calf	5	1 female calf	
2022			1		5		

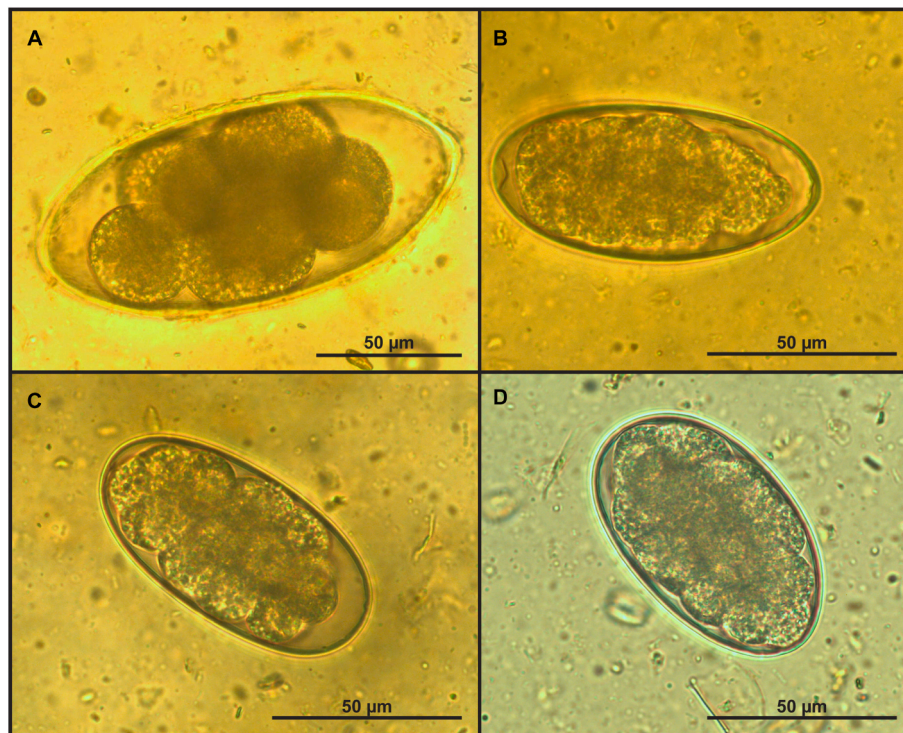


Fig. 1. Gastrointestinal nematode egg types (others than trichurids) recovered from faeces by flotation and enumerated by McMaster-method. A) *Nematodirus*, B) *Ostertagia*, C) *Cooperia*, and D) *Trichostrongylus*.



Fig. 2. Nematode larvae (live, total length 700 µm) hatched from larval cultures of European bison faeces, sampled March 2022. A. Intestinal cells clearly seen, short tail sheath extension. B. Larva with longer tail sheath extension.

serial and graded dilutions of ethanol, moved to xylene substitute, embedded in paraffin wax and sectioned (4 µm). Following deparaffinization the tissue was stained by haematoxylin, whereafter sections were mounted in Depex on microscope slide. All slides were studied in a Leica DM5000B compound microscope (Leica, Germany).

2.7. Molecular diagnosis

Ethanol conserved parts of adult and larval nematodes and the cestode were subjected to lysis using Proteinase K, DNA-purification, PCR with specific primers, product purification, sequencing and BLAST analysis. In brief, genomic DNA from parasites were isolated using the QIAamp DNA Mini Kit (cat.no. 51306, Qiagen, Denmark) according to the manufacturer's protocol. All PCR processes were performed in a 60 µl reaction using 6 µl of purified genomic DNA. Each reaction contained 1 mM DNTP mix, 1 µM of each of forward and reverse primers, 1.5 mM MgCl₂, 6 u BIOTAQ DNA Polymerase & 6 µl 10x NH₄-buffer (both cat.no. Bio-2160, Saveen & Werner ApS, Denmark), and

UltraPure™ DNase/RNase-Free Distilled Water (cat.no. 10977049, Thermo Fisher Scientific; Denmark) up to 60 µl. The PCR conditions consisted of an initial denaturation step at 95 °C for 5 min, 40 amplification cycles of denaturation at 95 for 30 s, annealing at primer specific temperature for 30sec, elongation at 72 °C for 2 min and a finally post-elongation step at 72 °C for 7 min. Primers for PCR and sequencing are listed in Table 2. PCR products were visualized by ethidium stained 2% agarose (cat.no. 10264544, Thermo Fisher Scientific, Denmark) gel electrophoresis and purified using illustra™ GFX™ PCR DNA and Gel Band Purification Kit (cat.no. 28-9034-71, VWR International A/S, Denmark). The purified PCR products were sequenced at Macrogen Europe B.V., Netherlands. The obtained sequences were assembled and analysed using the software CLC Main Workbench version 20.0.4.

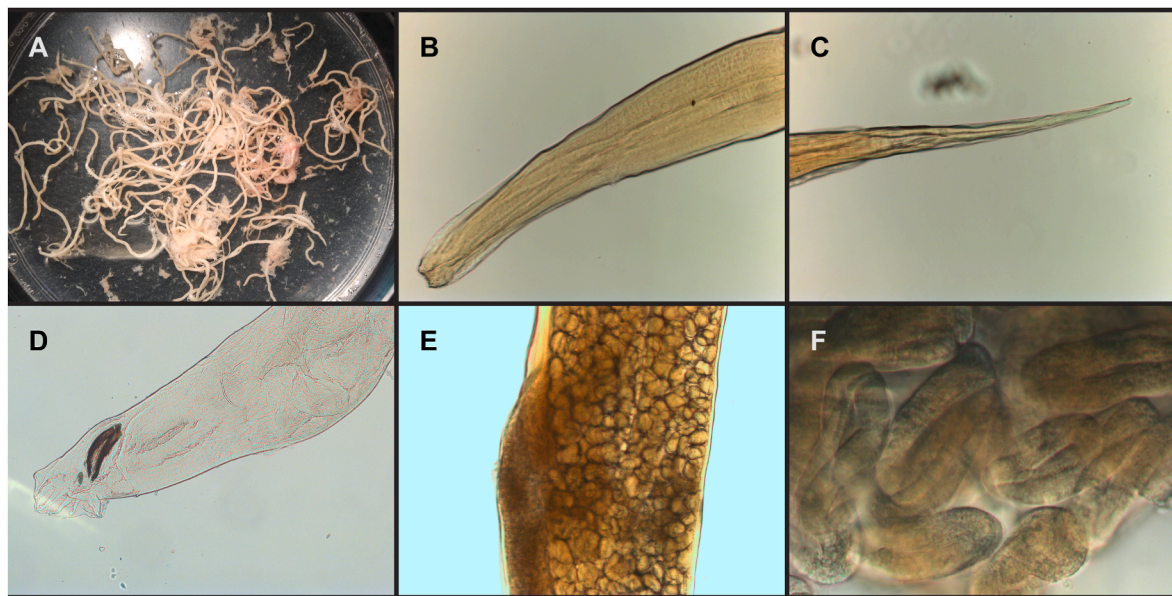


Fig. 3. Lungworms, *Dictyocaulus viviparus* recovered from the bronchi of a young bull of *Bison bonasus* found dead January 2017. A. Macroscopic appearance. B. Light microscopy LM of female worm (anterior). C. Light microscopy LM of female worm (caudal part). D. Male worm caudal part. E. Uterus with eggs in female lungworm. F. Embryonating eggs in female lungworm uterus.

Table 2

Primers and target regions used for PCR. Primers used for PCR and subsequent sequencing. All primers are read from 5' end to 3' end. T_a, T_e: Indicates annealing temperature and elongation time, respectively. The primer OsOs_F1 was designed for this study.

SpeciesGene	Forward primer Reverse primer	T _a T _e	Ref.
<i>Dictyocaulus viviparus</i> 18S rDNA	F1_SSU: AAGATTAAGCCATGCATGTC R2_SSU: ACCTTGTGTACGACTT	55 °C 105 s	[Pyziel, 2014]
<i>Dictyocaulus viviparus</i> ITS2	ITS2F: ACGTCTGGTT CAGGGTTGTT ITS2R: TTAGTTTCTTTCTCCGCT	56 °C 30 s	[Hoglund et al., 1999]
<i>Trichostrongylus axei</i>	OsOs_F1: AATCGCAATGGCTTGAACCG	55 °C	[This study; Gasser et al., 1993]
<i>Haemonchus contortus</i> ITS1 → ITS2	NC5: TTAGTTTCTTTCTCCGCT	60 s	
<i>Moniezia</i> sp. ITS1 → ITS2	BD1: GTCGTAACAAGGTTTCCGTA BD2: TATGCTAAATTCAGCGGGT	57 °C 105 s	[Galazzo et al., 2002]
<i>Moniezia</i> sp. COX1	COX-F: GATGTTTTCTTTACATTATCTGGTG COX-R: GCCACCACAAATCAAGTATC	53 °C 60 s	[Haukisalmi et al., 2004]
<i>Moniezia</i> sp. NAD1	MoNad1F: GTTTGCGTCTCGAAGGTAGAGTAATTC MoNad1R: CTCACCGAACCAAAAGCGGA	60 °C 105 s	[Haukisalmi et al. (2018)]

3. Results

3.1. Parasites isolated

Adult specimens of lungworms and a cestode were isolated from adult bison bull and a calf during necropsy. Identification was performed by morphological and molecular methods as described below. Additional identification of parasites was based on dung samplings. Flotation, sedimentation and Baermann methods recovering eggs and larvae showed, based on morphology, that a range of parasites within several phyla/genera occurred in the introduced European bison, including trematodes (*Fasciola hepatica* (liver fluke) and rumen fluke), *Dictyocaulus viviparus* (lungworm), GIN such as strongyles and trichurids (*Trichuris* and *Capillaria*). As judged by egg morphology (Fig. 1) it is suggested that strongyle genera such as *Nematodirus*, *Ostertagia*, *Cooperia* and *Trichostrongylus* were represented.

Larval cultures prepared from samples in June 2013, December 2015 and March 2022 were set up (Fig. 2 A and B). Identification of infective GIN larvae from the cultures was complicated by largely unknown genera/species in bison but a rough distribution in five out of seven samples from June 2013 indicated presence of *Ostertagia* spp. (21%)

(range: total 760–980 µm; sheath extension 45–85 µm), *Trichostrongylus axei* (20%) (range: total 660–850 µm; sheath extension 30–45 µm), *Cooperia oncophora* (14%) (range: total 740–1070 µm; sheath extension 80–115 µm), other cattle *Cooperia* spp. (12%) (range: total 720–880 µm; sheath extension 65–80 µm), *Haemonchus placei* (4%) and *Oesophagostomum/Chabertia* spp. (2%). A remaining proportion (27%) was in the range of 650–750 µm (length) with a range of 65–85 µm sheath extension which could be compatible with *Cooperia* spp. but the typical “eye” dots were not seen. These larvae were, however, in size compatible with *H. contortus* of ovine origin. There was very large variation in proportions between samples, and the two last samples were negative for GIN. In December 2015 only a single sample with a high faecal egg count (960 epg) was set up for larval culture. It revealed 9% *Ostertagia* spp. (range: total 790–811 µm; sheath extension 67–74 µm), 19% *C. oncophora* (range: total 815–911 µm; sheath extension 84–103 µm), and 39% other cattle *Cooperia* spp. (range: total 670–776 µm; sheath extension 62–83 µm) or alternatively *H. contortus*, as mentioned above. A proportion (33%) (range: total 611–655 µm; sheath extension 74–82 µm) could not be identified properly as these were shorter than both other *Cooperia* spp. and *H. contortus* and considerably longer than cattle *Bunostomum* spp.

3.2. Molecular diagnosis

Specific identification was performed by PCR and sequencing. Four nematodes recovered from bronchi of a calf and an adult bull (Fig. 3) showed 100% similarity (18S and ITS2) with *Dictyocaulus viviparus* (Table 3) isolated from European bison in Poland. One cestode recovered in a female calf was belonging to the genus *Moniezia*, as inferred by the histological sections of the proglottids. When performing molecular identification of the isolate by using PCR and sequencing the genus was confirmed. With regard to the species level, by targeting both nuclear and mitochondrial DNA, we found no clear segregation towards *M. benedeni* or *M. expansa* sequences obtained from Genbank, when using a variety of *Moniezia* species and species within Cyclophyllidea as outgroups in a phylogenetic analysis of ITS2 (data not shown). Thus, the obtained ITS sequence did not comply clearly with any expected species such as *M. benedeni* or *M. expansa* (Table 3). This was further supported by sequencing the mitochondrial genes COX1 and NAD1. The ITS2 contained an estimated 29 bp long stretch which all exhibited multiple nucleotides, which were designated N. Blasting the ITS2 resulted in 7 entries, all *M. expansa*, having identities above 90% but a poor coverage of 23% and thereby not conclusive. This might be due to indels and/or hybridization. However, the genus *Moniezia* identification was confirmed but the species level remain undetermined. A total of 13 Larvae recovered from larval cultures from eggs isolated from bison faeces were individually subjected to molecular diagnosis as well. PCR and sequencing of ITS1-5.8S-ITS 2 revealed 10 *Trichostrongylus axei* and 3 identical *Haemonchus contortus* (Table 3) among the gastrointestinal parasites. The 10 *T. axei* exhibited 6 genetic variations with identities ranging from 99.33% to 100% towards the GenBank acc.no. KR011271 from roe deer in Russia.

3.3. Prevalence and intensity

Trematode eggs were recovered during the first years. Liver fluke *Fasciola* eggs were found in 2013 only (prevalences 20–43% with mean intensities 1–18 egg per 5 g faeces), whereas rumen fluke eggs occurred also in 2015 (prevalences 8–71%, mean intensities per 5 g faeces). From 2015 no trematode eggs were noted until 2022 when one sample was

Table 3

Molecular identification of parasites isolated from European bison in the fenced forest area. Taxon, GenBank accession numbers for parasite isolates in this study, gene target region, and similarity with previously recorded GenBank data (accession numbers with host species indicated with geographic location of sample used for comparison).

Species found	Gene	Identification	Percentage	Origin
GenBank acc.nos.		Genbank acc.no.		
<i>Dictyocaulus viviparus</i> ON668047→ON668050	18S rDNA	KC771250 <i>Bison bonasus</i>	100	Poland
<i>Dictyocaulus viviparus</i> ON677959→ON677962	ITS2	KF007339 <i>Bison bonasus</i>	100	Poland
<i>Trichostrongylus axei</i> ON6779546→ON677955	ITS1 → ITS2	KR011271 <i>Capreolus capreolus</i>	99.33 to 100	Russia: Ryazan region
<i>Haemonchus contortus</i> ON677956→ON677958	ITS1 → ITS2	LS997563 MHco3(ISE)	100	Not available
<i>Moniezia</i> sp. ON677249	COX1	AY568213 <i>Rangifer tarandus</i>	89.54	Finland
<i>Moniezia</i> sp. ON677250	NAD1	KX121040 Buffalo calves	88.43	China
<i>Moniezia</i> sp. ON677945	ITS1- 5.8S	AY752651 <i>Rangifer tarandus</i>	90.25	Finland

found positive (Table 4).

The egg excretion (Fig. 1) of GIN other than trichurids indicated that, with a few exceptions, nearly all animals tested were positive (100% prevalence) throughout the study period. The worm load expressed as mean egg excretion reached generally a few hundred egg. Maximum counts reached 960 egg. Molecular identification (above) showed presence of *Haemonchus contortus* and *Trichostrongylus axei* (Table 3). Identification of nematodes based on egg and larval morphology is less precise, but presence of genera *Nematodirus* (Fig. 1A), *Ostertagia* (Fig. 1B), *Cooperia* (Fig. 1C) and *Trichostrongylus* (Fig. 1D) was suggested, although specific counts for the different taxons were not recorded. Trichurid/Enoplid eggs were found at moderate levels with a maximum prevalence for *Trichuris* spp. of 80% and intensities of 20–40 egg. The infection with lungworms, as judged from the number of larvae in 10 g faeces, showed that the prevalence of infection reached 100% in 2017 and then remained high. The highest intensities were found in 2018 and 2019 reaching a maximum level of 484 and 200 larvae per 10 g faeces, respectively (Table 4). Whenever it was possible to associate faecal samples with a bison calf they were found positive for lungworm *D. viviparus* (Table 4).

3.4. Mortality

Detailed data on mortality in time and space in the herd is not available. At the end of the study period a total of eleven animals (six males, four females, one new-born calf) were present. The mortality was particularly notable in 2017 and 2018 where several calves were found dead (Table 1). As around 31 animals were introduced or born in the area the overall mortality is estimated to near 66%. Reasons for death reported were in two cases associated with injuries. One bull was euthanized due to its bad constitution (associated with a high bronchial load of *D. viviparus*). Some animals were found dead and subjected to necropsy by a local veterinarian (Table 1). Other animals were merely found in a decaying state (Fig. 4).

4. Discussion

The large herbivores introduced into the fenced area originated from the Polish Bialowieza forests. The parasitic infections in European bison in that particular area has been investigated thoroughly through decades. A list of 88 parasite species recovered from these hosts was presented by Karbowski et al. (2014b a; b). It would therefore be expected that import of these hosts into other geographic areas also would include import of at least some of the listed parasites. Following initial establishment of the European bison into the recipient area on the island Bornholm, which not previously had been populated by this species, we obtained fresh faecal samples (from 2013 to 2022) and subjected these to copro-parasitological examination. The dung samples were found to carry eggs from a series of parasites, including two fluke species, the liver fluke *Fasciola* and a rumen fluke. It is not possible to discern, based on egg morphology alone, if the rumen fluke is *Calicophoron daubneyi* or *Paramphistomum cervi*. Therefore we here apply the term rumen fluke. In addition, we recognized one cestode (*Moniezia* sp.), lungworms (*Dictyocaulus viviparus*), and GIN (*Trichuris* sp., *Capillaria* sp., and strongyles). Based on molecular examination of individual larvae recovered from larval cultures *Trichostrongylus axei* and *Haemonchus contortus* were identified in 2022. It is most likely, as judged from egg and larval morphology, that the bison also host GIN genera like *Nematodirus*, *Ostertagia*, *Cooperia* and *Oesophagostomum/Chabertia* but this needs further molecular verification. The nematode *Ashworthius sidemi* was not identified. This is noteworthy as the species is considered particularly pathogenic to European bison (Osinka et al., 2010). The molecular analysis comparing sequences from nematodes recovered in this study with accessible GenBank data indicated a relation (high similarity 100% and 99.3) with parasites isolated in Poland (*Dictyocaulus*) and Russia (*Trichostrongylus*). The cestode affiliation was found less clear as the

Table 4

Details on eggs and larvae in faecal samples. Prev.: Prevalence of infection (%). Int.: Intensity expressed as eggs per gram faeces (for GIN), eggs per 5 g faeces (trematodes) and larvae per 10 g faeces (*Dictyocaulus*).

GIN: other gastrointestinal nematodes than trichurids. NT: not tested.

Parasite	No. of Samples	Fasciola hepatica		Rumen flukes		Dictyocaulus viviparus ^a		GIN (strongyles)		Trichurids		Moniezia sp.	
		Prev. %	Int. (Range)	Prev. %	Int. (Range)	Prev. %	Int. (Range)	Prev. (%)	Int. (Range)	Prev. (%)	Int. (Range)	Prev. %	Int. (Range)
2013 (1)	7	43	18.3 (4–38)	71	39.2 (4–137)	14	4	71	112 (80–160)	28	20.5	0	0
2013 (2)	5	20	1 (1)	40	2.5 (2–3)	NT	NT	60	260 (180–380)	20	60	0	0
2013 (3)	7	29	1 (1)	57	2.8 (1–6)	NT	NT	100	260 (100–520)	42	146	0	0
2015	12	0	0	8	4	NT	NT	100	185 (4–960)	8	20	0	0
2017	4	0	0	0	0	100	6.5 (2–12)	75	53 (20–100)	0	0	0	0
2018	9	0	0	0	0	66	95.8 (5–152)	100	146 (40–460)	22	20	11	20
2019	4	0	0	0	0	25	200 (200)	100	130 (80–240)	20	20	0	0
2020	3	0	0	0	0	66	184 (50–517)	66	183 (180–280)	0	0	0	0
2021	5	0	0	0	0	60	1 (1–2)	100	144 (20–400)	80	240	0	0
2022	5	20	1 (1)	0	0	40	1 (1–2)	100	95 (60–140)	0	0	0	0

^a Faecal samples positively associated with bison calves were positive for lungworm larvae.



Fig. 4. Calf found dead and mummified under a Norway spruce in the fenced area. Hind legs, fore legs, ribs and frontal horns are noted protruding from the skin covering.

sequence similarities were lower (88.43–90.25%) with nearest affiliation with isolates from China and Finland, respectively. Fluke eggs in faeces from older animals were still observed in 2015 but not in the calves, which indicated that the life cycle of these trematodes, dependent on intermediate host snails, did not establish in the study area. The analysis of the bison diet based on ingested herbs (Hartvig et al., 2021) showed that raspberry *Rubus idaeus* dominated the diet, which indicated foraging on dry land. However, a series of plant species associated with meadows and other wetland habitats, which are habitats of potential intermediate hosts (lymnaeid and planorbis snails), were also recorded (Hartvig et al., 2021). Future malacological surveys should clarify if these gastropods occur in the area. The re-occurrence of a light *Fasciola* infection (one egg in one faecal sample) in 2022 suggests re-introduction

or that the infection could have persisted at a low level during the years, but the reservoir host is at present unknown. The cestode (*Moniezia* sp.), lungworms (*Dictyocaulus viviparus*), gastrointestinal nematodes (*Trichuris*, *Capillaria*, *Trichostrongylus*, *Haemonchus*) became established also in the calves. The cestode life cycle includes oribatid mites (acting as intermediate hosts) indicating presence of this chelicerate type in the forest. Due to the low cestode prevalence and intensity this parasite type probably has no or only minor effect on the performance of European bison in the fenced area, as this is the case with sheep and cattle in general (Bowman, 1999). The nematodes found do not employ intermediate hosts and showed the ability to propagate and spread effectively to both adults and calves. Gastrointestinal parasites found in Polish bison from the region of origin are considered pathogenic to the host

(Osinska et al., 2010). In addition, the high occurrence of lungworms in calves call for increased awareness as their pathogenicity is well established (Demiaszkiewicz et al., 2009). Thus, it cannot be excluded that the parasitic infections observed in the study area have influenced the overall mortality in the flock. Thus, the prevalence and intensity of lungworm infection reached a high level in the years 2017–2018, the period in which the main mortality was observed among calves. The natural mortality among European bison in the Polish part of the Białowieża forest, recorded during the period from 1959 to 2002, was found associated with 13 different diagnoses. Parasitic diseases accounted for 4.9–8.1% of causes of death, and especially lungworms were considered the main causative pathogens (Krasinska and Krasinski, 2007). Apart from helminths also other parasites, such as protozoans including *Neospora* (Bien et al., 2010), *Eimeria* (Pyziel et al., 2014, 2020), bacteria (Krasinska and Krasinski, 2007) and virus (Haigh et al., 2002) may challenge the health, reproduction, welfare and survival of European bison in Poland. In addition various arthropod parasites, including mites, carried by European bison should be noted (Izdebska, 2006). This calls for further investigations supplementing the helminth studies with focus on these other pathogens (Haigh et al., 2002). In this context also ticks (e.g. *Dermacentor reticulatus* and *Ixodes ricinus*) should be included as they carry Tick Borne Encephalitis Virus (TBEV) as documented by samplings directly from Polish European bison in the Białowieża forest, known to be endemic for TBEV (Biernat et al., 2016). The present Danish survey is preliminary, but it points to presence of helminths in the introduced European bison and suggests that the infections may explain a part of the mortality. The investigation is entirely observational but the overall mortality recorded and the well-described pathogenicity of lungworms in European bison suggest that anthelmintic treatment, or other means of control, may be beneficial and contribute to a higher survival and thereby elevated success of the European bison population. Deworming programmes have been used for captive European bison in Poland (Kołodziej-Sobocinska et al., 2018). However, for practical management in the field, it may be worth to consider that studies on the use of avermectins, administered both as pour-on and injection preparations, have documented a high efficacy against lungworm and GIN in *Bison bison* (Woodbury and Lewis, 2011; Eljaki et al., 2016). These precautionary measures should be addressed before translocation projects between different geographic regions, as European bison may act as reservoir of e.g. several cervid parasites (Drozd et al., 2002, 2003). Recent comprehensive analyses, presented from the Polish area of *Bison bonasus* distribution, point to the function of this host species as carrier of 12 species of gastrointestinal parasites with cervids as the natural hosts (Karbowiak et al., 2014 a; b). Transplanting European bison to recipient regions, in which these parasites did not occur previously, introduce a risk of transferring cervid parasites to hitherto naïve host populations. The impact of such transfers on populations of cervids in the recipient, e.g. roe deer (Drozd et al., 2003), should be further elucidated. It can therefore be recommended to perform a baseline investigation before introduction of alien species. In conclusion, nature restoration projects involving endangered large herbivores, such as the European bison, may impose a risk of parasitic disease not only to the animal released but also to the endemic animal populations. Further, the population may benefit from monitoring and perhaps timely and controlled interventions against various diseases including the pathogenic parasites. General health improvement and thus survival of the animals based on improved health care is likely to improve welfare and contribute to social acceptability.

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Declaration of competing interest

The authors declare that they do not have any conflict of interests.

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The authors are all employed at the University of Copenhagen.

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