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Endoparasite loads and the efficacy of conventional anthelmintics against gastrointestinal nematodes in captive European bison

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ABSTRACT

Although little information exists on the efficacy of deworming in wild ruminants, gastrointestinal nematodes have been found to demonstrate increasing drug resistance. The spread of drug-resistant strains may be increased by transmission among livestock and susceptible wildlife species, thus posing a potential threat to endangered species, such as the European bison.

The aim of the study was twofold: to identify the parasite loads in captive European bison with the use of coprological techniques, and to test the influence of other nearby ungulates on the richness of bison parasitofauna. Additionally, the efficacy of deworming procedures against gastrointestinal nematodes in bison was evaluated. The survey was based on a coprological investigation of 285 fecal samples from 156 European bison in 15 enclosures.

The parasitofauna of the captive European bison was consistent with those of free-ranging populations. The highest prevalence was noted for *Eimeria* spp. oocysts (60.7%), strongyle eggs (50.9%), *Fasciola hepatica* eggs (13.1%), *Dictyocaulus viviparus* larvae (12.3%) and *Trichuris* sp. Eggs (9.47%). Moreover, the close proximity of other ungulate species resulted in a higher diversity of parasite species.

In all cases, deworming with albendazole, fenbendazole and ivermectin proved to be ineffective against strongylids and *Trichuris* sp. The results of fecal egg count reduction test (FECRT) ranged from 37.2 to 99.6% (95% CI <90%) for albendazole; values >95% (95% CI = 41–100) were noted for fenbendazole, and FECRT ranged from 63.2 to 97.5 (95% CI = 0–99) for ivermectin.

As the results of anthelmintic treatment are unsatisfactory, it seems justified to continue study in this area. Our study is the first large-scale attempt to evaluate the efficacy of anthelminthics in captive European bison. The potential sharing of parasite species between bison and other ungulates should also be further investigated from the perspective of minimizing the risk of the spread of drug-resistant parasite strains.

1. Introduction

The last decade has seen an increase in cases of gastrointestinal nematode resistance to anthelminthics worldwide (Kaplan and Vidyashankar, 2012; Vercruysse et al., 2018). In addition to infecting livestock and causing economic losses to livestock farming, nematodes can also be transmitted to wild host species, thus increasing their spread, including that of drug-resistant strains (Barone et al., 2020). This phenomenon could also affect the diversity of wild ruminants (Williams et al., 2002; Barone et al., 2020) and pose a potential threat to endangered species such as European bison, *Bison bonasus*, which is listed by the IUCN as a near-threatened species (IUCN, 2022). Very little information exists on the efficacy of deworming in wild ruminants. One key consideration is that long-term, routine deworming treatments are associated with drug resistance (Kaplan and Vidyashankar, 2012; Pyziel et al., 2018a).

According to recent data, the global European bison population

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currently comprises 9500 individuals, of which 25% live in captivity (Raczyński and Bołbot, 2022). Captive animals are important for the survival of the species as they are often used in reintroduction programs and conservation strategies (Tear et al., 1993; Mathews et al., 2006); this is also the case for European bison (Olech and Perzanowski 2022). All surviving European bison were restored from a small group of only 12 founders. This has resulted in a dramatic decrease in their genetic variation, making them particularly vulnerable to diseases, including parasitoses (Olech and Perzanowski, 2002; Kita and Anusz, 2006; Krasińska and Krasiński, 2007; Demiaszkiewicz et al., 2009). Parasitic invasions are likely to cause severe symptoms, e.g., hemorrhagic diarrhea or bronchitis, and may pose major problems in captive populations (McCallum and Dobson, 1995; Jolley and Bardsley, 2006; Pyziel et al., 2018b).

Parasitic fauna characteristic of cervids, cattle and small ruminants are commonly found in European bison, which foster parasite exchange and the transmission of anthelmintic resistance mutations between domestic and wildlife species (Barone et al., 2020). In such cases, the risk of infection is strongly influenced by direct or indirect contact between European bison, livestock, and other free-ranging animals. In European bison, the most prevalent endoparasites are nematodes from the Trichostrongylidae family, as well as *Trichuris* sp. and *Fasciola hepatica* (Karbowiak et al., 2014b). Bison most commonly share parasites with cattle (*Bos taurus*, e.g., *F. hepatica*, *Dictyocaulus* spp.), followed by roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*, e.g., *Spiculopteragia asymmetrica*, *Cooperia surnabada*); however, cases of cross-transmission have also been recorded with moose (*Alces alces*) (e.g., *Parafasciolopsis fasciolaemorpha*) (Karbowiak et al., 2014a, 2014b).

Stephens et al. (2019) indicate that the risk of infection in endangered species may be significantly increased by proximity to other species susceptible to the same parasites. We hypothesized that parasite diversity in European bison could also be increased by close proximity to other ungulate species that are susceptible to infestation with the same parasites.

The aims of the study were to identify the species of parasitofauna present in captive European bison in Poland and evaluate the efficacy of deworming procedures against gastrointestinal nematodes in the European bison. The study also evaluated the impact of other nearby ungulates on the richness of the parasitofauna in European bison.

2. Material and methods

2.1. Material collection and area of the study

The study was performed in 15 European bison enclosures in Poland in 2018–2020, including six breeding centers (Białowieża Breeding Center, Smardzewice Breeding Center, Niepołomice Breeding Center, Gołuchów Breeding Center, Pszczyna-Jankowice Breeding Center, Wolisko Breeding Center), five zoos (Bałtów zoo, Warsaw zoo, Ustroń zoo, Pszczyna zoo and Poznań zoo) and four enclosures (Białowieża enclosure, Kiermusy enclosure, Międzyzdroje enclosure, Muczne enclosure) (Fig. 1). The study was conducted on animals of both sexes in the age range <1–17 years (Tables 4–7), but there was no division into age groups.

Captive cervids such as red deer (*Cervus elaphus*), roe deer (*Capreolus*), fallow deer (*Dama dama*) and moose (*Alces alces*) were present in the vicinity of the Białowieża, Pszczyna-Jankowice and Gołuchów breeding centers and the Bałtów, Międzyzdroje, Pszczyna and Ustroń enclosures. The only neighboring equid species was the Polish konik (*Equus Ferus Caballus*) in the case of Gołuchów Breeding Center and Białowieża Enclosure. As for bovids, European mouflon (*Ovis aries musimon*) were kept in close proximity to the European bison in Pszczyna enclosure and Ustroń zoo, and scimitar oryx (*Oryx dammah*) was kept close to bison in Warsaw zoo (Table 1).

The proximity of other ungulate species was assessed based on the distance between the enclosures. Neighborliness was recognized in cases where enclosures of different animal species were bordered by a fence or were located in the same part of the reserve, but separated by a path or road. Locations where no other ungulate species were kept, or where the European bison were located in a distant part of the center, were considered not to meet these criteria. Neither the Białowieża, Smardzewice, Niepołomice or Wolisko Breeding Centers, nor the Kiermusy and Muczne enclosures or Poznań zoo were positioned near cervids, equids or bovids (Fig. 1).

A total of 285 fecal samples were collected. Collection was performed in the spring and fall seasons to account for seasonal fluctuations in parasite activity and the timeframes of annual deworming treatments; it was limited to individuals which had been in a given enclosure for at least one month.

Sampling was carried out under conditions without heavy rainfall and when identification of the studied individual was possible. Feces samples were collected from the top of freshly passed deposits on the

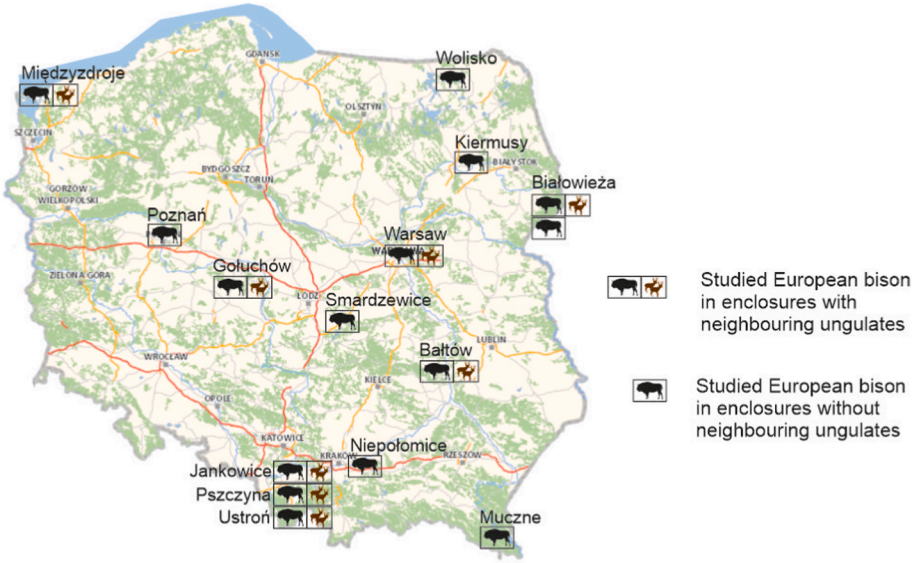

Fig. 1. Location of coproscopically examined European bison enclosures and other ungulate species in the vicinity of these enclosures in Poland.

Table 1
Number of faecal samples examined in European bison enclosures and other ungulate species in the vicinity of these enclosures.

Location of enclosure	No. of examined faecal samples	Red deer (*Cervus elaphus*)	Roe deer (*Capreolus*)	Fallow deer (*Dama*)	Moose (*Alces alces*)	Polish konik	European mouflon (*Ovis aries musimon*)	Scimitar oryx (*Oryx dammah*)
Enclosures								
Białowieża	23	+	+		+	+		
Kiermusy	2							
Międzyzdroje	9	+	+					
Muczne	53							
Breeding centers								
Białowieża	21							
Gołuchów	27			+		+		
Niepołomice	10							
Pszczyna-Jankowice	47	+	+	+				
Smardzewice	6							
Wolisko	9							
ZOOs								
Bałtów	7			+				
Poznań	5							
Pszczyna	17	+		+			+	
Ustroń	17	+					+	
Warsaw	32							+

ground. These were placed in 50 ml plastic tubes at 4 °C and promptly transferred to the laboratory for coprological investigation. The samples were collected with the support of the staff at the breeding center or enclosure and with the consent of the authorities of the centers or enclosures; they were examined immediately, no later than 2 days after collection. Due to the nature of the study, approval was not required from local ethical commissions, according to Polish law.

2.2. Coprological investigation

All 285 samples, collected from 156 European bison, were examined by a modified McMaster technique in sucrose solution (SG = 1.27) to detect nematode eggs and Eimeria oocysts. The eggs were then identified to the species, genus or family level (Shorb, 1939; Skryabin et al., 1991; Taylor et al., 2007; Delling et al., 2020). The oocysts were determined to the genus level according to Duszynski and Wilber (1997) and Pyziel et al. (2014, 2019). In total, 268 samples were tested for lungworm larvae using the Baermann technique (Eysker, 1997); 259 were tested for trematode eggs using the sedimentation method (Pyziel et al., 2014a). Thus, only 259 of all 285 samples were investigated using all three coprological methods. For each method, 3 g fecal samples were used. The samples were investigated using a LAB 40 light microscope (OPTA-TECH, Poland) at × 100–400 magnification for the McMaster technique, a stereoscopic microscope (Delta Optical) at × 100 magnification for the Baerman technique, and × 40 magnification for the sedimentation method.

2.3. Anthelmintic efficacy

In eight European bison reserves in spring or autumn, the efficacy of treatment against gastrointestinal nematodes with three groups of anthelmintics was evaluated: albendazole, fenbendazole (benzimidazole) and ivermectin (macrocyclic lactones), either alone or combined with praziquantel and levamisole (imidazothiazole).

The animals were treated with the following: albendazole (10 mg/kg P.O.; Valbazen 10%, Zoetis, Belgium) or fenbendazole (10 mg/kg P.O.; Fenbenat 40 mg/g, VETOS-FARMA, Poland) for five consecutive days; ivermectin (0.3 mg/kg P.O.; Iwermektyna Vetos-Farma 0.6g/100g, VETOS-FARMA, Poland) administered once, or levamisole (7.5 mg/kg P.O.; Levamol 5%, Vetoquinol Biowet, Poland) administered once.

In Ustroń and Warsaw zoos, as well as in Niepołomice Breeding Center, the body weight of each European bison was estimated, and the dosages of anthelmintics were mixed with concentrate and administered individually. In Białowieża and Gołuchów Breeding Centers, Poznań and Pszczyna zoos, and Białowieża and Muczne enclosures, the animals were divided into groups based on the estimated body weight, and the drugs were administered by mixing them in pelleted feed. One day before deworming, the animals did not receive concentrate feed.

The Fecal Egg Count Reduction Test (FECRT) was used to determine the efficacy of anthelminthic treatment (Coles et al., 1992, 2006). FECRT was performed on 57 individual fecal samples using a modified McMaster technique (Taylor et al., 2007). Testing was performed one day before planned deworming (pretreatment control) and after anthelminthic treatment (McKenna, 1994). Only samples with egg counts (EPG) of 100 or greater were included in the study, as recommended for cattle (Coles et al., 2006); altogether, 35 samples were qualified for FECRT analysis and 22 were excluded.

The FECRT protocol for cattle was modified due to the low number of individuals in the enclosures. All animals were included in the study groups; no control groups were created, and the number of samples qualified for the FECRT was lower than recommended by WAAVP. To perform calculations on a larger group of samples, the overall FECRT analysis was performed taking into consideration all animals dewormed with the same drug in all enclosures. The overall FECRT score was calculated for each analyzed anthelmintic: albendazole, fenbendazole and ivermectin.

Anthelmintic efficacy was evaluated for both strongylids (30 samples) and *Trichuris* sp. (five samples). Post-treatment collection was performed depending on the drug used: benzimidazole 8–10 days after deworming, imidazothiazole 3–7 days later, macrocyclic lactones 14–17 days later.

Reduced deworming efficacy was determined according to the WAAVP guidelines: FECRT <95% and lower limit of 95% CI <90% (Coles et al., 1992). The analysis was performed using the eggCounts-2.3 module on R version 3.6.1, developed at the Department of Mathematics at the University of Zurich (Wang and Paul, 2018; Wang et al., 2018). Data were analyzed using a two-sample paired model, i.e., pre- and post-treatment egg counts, for each individual with a correction factor of 50.

2.4. Statistical analysis

2.4.1. Impact of other ungulate species on parasitofauna of the European bison

The statistical analyses only included the fecal samples obtained from European bison before deworming (n = 207). The influence of the proximity of other ungulates on the presence of parasites in European bison was tested using two generalized linear models with a Poisson distribution and a log link function. The analysis was divided into two parts based on the results of the McMaster technique. The first model analyzed the number of *Eimeria* species in samples from the European bison in the enclosures: the number of species found in each sample from an individual was the dependent variable, and the explanatory variables were season and the presence of other ungulates in the vicinity of European bison's enclosure. In the second model, the same analysis was performed, but the dependent variable was the number of genera of all parasites detected by the McMaster technique, *Eimeria* spp. included, with no distinction by species, i.e., if two different species of *Eimeria* occurred, only one genus was recorded. The sex and age of the European bison specimens were not included due to insufficient individual data.

Both models included two independent variables, *viz.* proximity and season, and the interaction proximity*season. Model selection was performed by analyzing all variants of the model, including the null model (Burnham and Anderson, 2002). Finally, we ranked the models according to their AIC values and used the highest ranked model with the lowest AIC.

3. Results

3.1. Coprological findings

The McMaster technique detected Oocysts of *Eimeria* spp. and eggs of gastrointestinal nematodes belonging to the order Strongylida, including representants of the superfamily Strongyloidea and Trichostrongyloidea (strongylids), the superfamily Ascaridoidea, as well as the genera *Trichuris*, *Nematodirus* and *Aonchoteca*, and the genus *Moniezia*. Larvae of lung nematodes from the genus *Dictyocaulus* were identified with the Baermann method. Trematode eggs of the genus *Fasciola* and family Paramphistomidae were found by sedimentation (Table 2).

The highest prevalence was noted for *Eimeria* spp. oocysts (60.7%), strongyle eggs (50.9%), *Fasciola hepatica* eggs (13.1%), *Dictyocaulus viviparus* larvae (12.3%) and *Trichuris* sp. Eggs (9.5%). The prevalence of other parasites did not exceed 4% (Table 2). The highest median oocyst/egg per gram (OPG/EPG) scores were obtained for *Eimeria* spp. (OPG = 250) and strongylids (EPG = 200), while the highest ranges were noted for *Trichuris* sp. (EPG = 50–13000), *Eimeria* spp. (OPG = 50–6600) and strongylids (EPG = 50–2850) (Table 2).

The enclosures exhibiting the highest diversity of parasitic genera in the European bison were Pszczyna-Jankowice and Gołuchów (eight genera), followed by Wolisko, Białowieża Breeding Center and Białowieża enclosure (six genera). Eggs of *Moniezia* sp. were found only in Gołuchów and Pszczyna-Jankowice, whereas eggs of Ascaridoidae were found in animals kept in both Poznań Zoo and Białowieża enclosure.

Oocysts of eleven species of *Eimeria* were identified (Table 3). The most prevalent were *Eimeria bovis* (49.8%), *E. auburnensis* (18.6%) and *E. subspherica* (11.6%), whereas the highest medians were noted for *E. bovis* (OPG = 200) and *E. alabamensis* (OPG = 150). The greatest ranges were found for *E. bovis* (OPG = 50–6350) and *E. alabamensis* (OPG = 50–1650) (Table 3) The highest diversity of *Eimeria* species was observed in Pszczyna-Jankowice (nine species) and Bałtów and Muczne (seven species). Kiermusy was the only enclosure where no coccidia were noted. Detailed results for each enclosure are available in the supplementary material (Supplementary material 1).

3.2. Anthelmintic efficacy

3.2.1. Fecal egg count reduction test (FECRT)

Two benzimidazole drugs were used: *viz.* fenbendazole in three enclosures and albendazole in five. Ivermectin, a macrocyclic lactone, was used in two breeding centers (Tables 4–7). Levamisole, an imidazothiazole, was used in one location; in this case, the egg counts were lower than 100 EPG and the samples were excluded from the FECRT analysis.

In all cases (four reserves, 18 samples), deworming with albendazole was not efficient against strongylids, as shown by the FECRT results, which ranged from 37.2 to 99.6% (95% CI <90%) with a mean value of 48% (95% CI = 17–49) (Table 4). A similar result was obtained with the use of fenbendazole in the Białowieża and Gołuchów Breeding Centers, with FECRT results of >95% (95% CI = 41–100; 47–100, respectively). Taking these results together, and adding the single result from the Niepołomice Breeding Center, the total FECRT score was 99.8% (95% CI = 77–100) (Table 5).

The last examined anthelmintic, ivermectin, was also not effective against strongylids in the two reserves where it was used (five samples). The FECRT scores were 97.5 (95% CI = 41–99) in the Białowieża Breeding Center and 63.2% (95% CI = 0–57) in the Gołuchów Breeding Center. The overall FECRT score for ivermectin calculated from all enclosures was 76% (95% CI = 8–76) (Table 6).

Albendazole was also ineffective against *Trichuris* sp., with a FECRT score of 51.7% (95% CI = 19–52) in Warsaw Zoo and 99.5% (95% CI = 30–100) in the Muczne enclosure. Its mean FECRT score was 67.3% (95% CI = 47–67) (Table 7).

Of the twelve analyzed anthelmintic treatments, only five scored FECRT >95% and none reached the threshold of 95% CI >90%. Among the detected helminths, *F. hepatica* eggs, Paramphistomidae eggs and

Table 2
Coprological findings in European bison after examination by modified McMaster technique (n = 285), Baerman technique (n_1 = 268) and sedimentation method (n_2 = 259).

	McMaster method							Baerman technique	Sedimentation method	
	Trichuris sp.	*Eimeria* spp.	Trichostrongyloidea	*Nematodirus* sp.	*Moniezia* sp.	*Capillaria* sp.	Ascaridoidea	*Dictyocaulus viviparus*	*Fasciola hepatica*	Paramphistomidae
Prevalence (%)	9.47	60.70	50.88	2.46	0.70	1.75	0.70	12.31	13.13	3.86
Mean EPG/OPG/LPG	1601.85	538.29	385.52	100.00	150.00	140.00	75.00	9.55	5.38	3.10
SD	3464.64	865.70	453.19	70.71	141.42	74.16	35.36	11.46	5.92	2.42
Median EPG/OPG	100.00	250.00	200.00	50.00	150.00	150.00	75.00	6.00	3.00	2.00
Range EPG/OPG	50-13,000	50-6600	50-2850	50–200	50–250	50–250	50–100	1–53	1–27	1–8

EPG/OPG/LPG: eggs/oocyst/larvae count per gram.

Table 3
Eimeria oocysts findings in European bison after examination by modified McMaster method ($n = 285$).

	E. alabamensis	E. auburnensis	E. bovis	*E. brasiliensis*	E. bukidnonensis	E. canadensis	E. cylindrica	E. ellipsoidalis	E. pellita	E. subspherica	E. zuernii
Prevalence (%)	1.05	18.60	49.82	1.75	0.35	1.05	0.70	2.11	6.32	11.58	8.77
Mean OPG	133.33	221.70	506.87	60.00	250.00*	66.67	50.00	100.00	100.00	119.70	72.00
SD	28.87	300.08	903.51	22.36		28.87	0.00	44.72	127.19	99.95	45.83
Median OPG	150.00	100.00	200.00	50.00		50.00	50.00	100.00	50.00	100.00	50.00
Range OPG	100–150	50–1650	50-6350	50–100		50–100	50,00	50–100	50–550	50–450	50–250

OPG: oocyst count per gram; *result from only one sample.

Table 4
Efficacy of albendazole against Strongylidae infection in European bison.

Location of enclosure	Sex	Age [years]	No. of examined faecal samples	No. of faecal samples with EPG ≥100	FECRT %	95%CI	Efficacy of deworming
Ustroń zoo	females males	2, 12 17	3	2	94.3	10–96.8	inefficient
Pszczyna zoo	females males	7, 11, 9 2, 2, 7	6	6	37.2	0–33	inefficient
Ustroń zoo	females males	2 <1, 17	3	3	60.2	0–55	inefficient
Białowieża enclosure	females males	8, 8, 10, 16 5	5	4	90.8	55–92	inefficient
Muczne enclosure	females males	1, 1, 9, 9, 11, 11 1, 1, 11	9	3	99.6	47–100	inefficient
Total			26	18	48.1	17–49	

Table 5
Efficacy of fenbendazole against Strongylidae infection in European bison.

Location of enclosure	Sex	Age [years]	No. of examined faecal samples	No. of faecal samples with EPG ≥100	FECRT %	95%CI	Efficacy of deworming
Białowieża Breeding Center	females males	8, 8, 10, 16 5	5	3	99.5	41–100	inefficient
Gołuchów Breeding Center	females males	11, 11 1, 1, 3, 11	6	3	99.6	47–100	inefficient
Niepołomice Breeding Center	females males	 10	1	1	–	–	inefficient
Total			12	7	99.8	77–100	

Table 6
Efficacy of ivermectin against Strongylidae infection in European bison.

Location of enclosure	Sex	Age [years]	No. of examined faecal samples	No. of faecal samples with EPG ≥100	FECRT %	95% CI	Efficacy of deworming
Białowieża Breeding Center	females males	1, 1, 1, 1.5 1, 1	6	3	97.5	41–99	inefficient
Gołuchów Breeding Center	females males	1, 12, 12 4, 12	5	2	63.2	0–57	inefficient
Total			11	5	76	8–76	

Table 7
Efficacy of albendazole against *Trichuris* sp. infection in European bison.

Location of enclosure	Sex	Age [years]	No. of examined faecal samples	No. of faecal samples with EPG ≥100	FECRT %	95%CI	Efficacy of deworming
Warsaw ZOO	females	<1, 1, 1.5, 3, 8.5, 8.5	8	3	51.7	19–52	inefficient
	males	2.5, 11.5					
Muczne enclosure	females males	1, 1, 9, 9, 11, 11 1, 1, 11	9	2	99.5	30–100	inefficient
Total			17	5	67.3	47–67	

D. viviparus larvae were noted in 14%, 5.3% and 5.3% of samples, respectively, before deworming, and in 8.7%, 6.5% and 6.4% of samples following treatment, but the efficacy of treatment against trematodes was not evaluated in this study.

3.3. Impact of other ungulate species on parasitic fauna in European bison

The European bison kept near to other ungulates showed an average number (1.5) of parasite types/genera. This value was significantly higher ($p = 0.031$) than in the European bison without ungulate neighbors (mean = 1.2 parasite types).

In addition, European bison kept close to other ungulate species also demonstrated an increased diversity of *Eimeria* species (Fig. 1) in autumn ($p = 0.000$) and winter ($p = 0.035$). In these two seasons, the mean number of parasite species was higher than 1.5 in enclosures with neighboring ungulates, while in enclosures without neighboring ungulates it was half the value (Fig. 2). In contrast, no differences were observed in spring ($p = 0.740$), when European bison in both sets of enclosures had a mean score of 0.8.

4. Discussion

The present study is the first large-scale attempt to evaluate the efficacy of anthelmintics in captive European bison. In all cases analyzed, based on WAAVP criteria, deworming proved to be ineffective for both strongylids and *Trichuris* sp. when using albendazole, fenbendazole and ivermectin. Therefore, the FECRT results in our study indicate the possible development of drug resistance in gastrointestinal nematodes in Polish enclosures. Previously, the only available data on FECRT examination in European bison was obtained in Avesta Visentpark in Sweden. The findings suggest that fenbendazole has reduced efficacy against strongylids, particularly *H. contortus* (Pyziel et al., 2018a). In addition, a study in Springe, Germany reported the presence of fenbendazole-resistant *H. contortus*, as well as suspected macrocyclic lactone resistance, although the latter was not confirmed (Springer et al., 2022). The low efficacy of albendazole against *Trichuris* sp. in our study might be a result of the generally low effectiveness of benzimidazoles in relation to this genus (Borgsteede, 1979; Hansen et al., 2014; Adegnika et al., 2015).

The FECRT protocol applied in our study in European bison differed from the standard WAAVP protocol recommended in livestock (Coles et al., 1992, 2006), thus the results should be interpreted with caution. From a total of 2500 European bison living in Poland, only about 200 specimens are kept in captivity (Raczyński and Bołbot, 2022). Therefore, deviations from the basic WAAVP guidelines included the small group sizes of the examined animals, thus there were no control groups and low pretreatment EPG values. Nevertheless, local parasite populations seem to have drug-resistant traits, which highlights the need for further research. It is reasonable to introduce preventive measures such as enclosure rotations and differentiated deworming protocols, and every newly imported animal should be treated as being potentially infected with drug-resistant parasites (Conder and Campbell, 1995; Gałązka et al., 2022). Drug resistance may develop for various reasons in captive non-domestic animals, such as inaccurate determination of body weight, which results in incorrect anthelmintic drug doses; in addition, individual drug administration and ensuring complete dosage intake can also be problematic, and there may be limited possibilities for enclosure rotation (Conder and Campbell, 1995; Coles et al., 2006).

The parasites' eggs, oocysts and larvae detected during our study match data on species diversity and the prevalence and intensity of parasitic infestation in European bison in central Europe (Balčiauskas, 1999; Forte et al., 2012, 2012a, 2012b;b; Yurchanka and Anisimava, 2015), but most available reports concern free-living populations. The highest prevalence was found for coccidia, with *E. bovis* as the most common species, which has also been commonly observed in European bison in previous studies (Pyziel et al., 2011, 2018a; Demiaszkiewicz et al., 2014, 2018, 2020, 2022; Klich et al., 2021). The high values of OPG for *Eimeria* spp. noted in our study may be a result of a range of individual and environmental factors, including limited or no enclosure rotation in captive European bison, the age of the examined animals, increased oocyst production in infected but subclinical animals, and high animal density in enclosures (Larsson et al., 2006; Pyziel et al., 2011). The presence of eggs of *F. hepatica* varied in both captive and free-living animals, depending on the examined populations (Demiaszkiewicz et al., 2014, 2022), while the prevalence of strongyle and *Trichuris* sp. Eggs and *D. viviparus* larvae in our study was generally lower in comparison to free-living European bison (Demiaszkiewicz et al., 2014, 2022). The examined animals were in good condition, were kept in small herds, and were regularly dewormed, therefore they may have demonstrated strong resistance to parasitic infections, which could have resulted in lower fecal egg counts (Medzhitov et al., 2012; Budischak et al., 2018; Roy and Kirchner, 2000). On the other hand, captive European bison might be genetically more susceptible to pathogens (Olech and Perzanowski, 2002; Krasińska and Krasiński, 2007). As such, living in restricted spaces, often with high population densities, could favor parasite transmission and lead to development of disease symptoms (Olech and Perzanowski, 2002; Kołodziej-Sobocińska et al., 2018).

Furthermore, our results suggest that the diversity of parasite species infecting European bison may be positively influenced by the presence of other ungulate species in neighboring enclosures. Cross-transmission of parasite species between European bison and other wild or domestic ruminants has previously been reported (Dróżdż, 1961; Dróżdż et al., 2003; Karbowiak et al., 2014a, 2014b; Walker and Morgan, 2014;

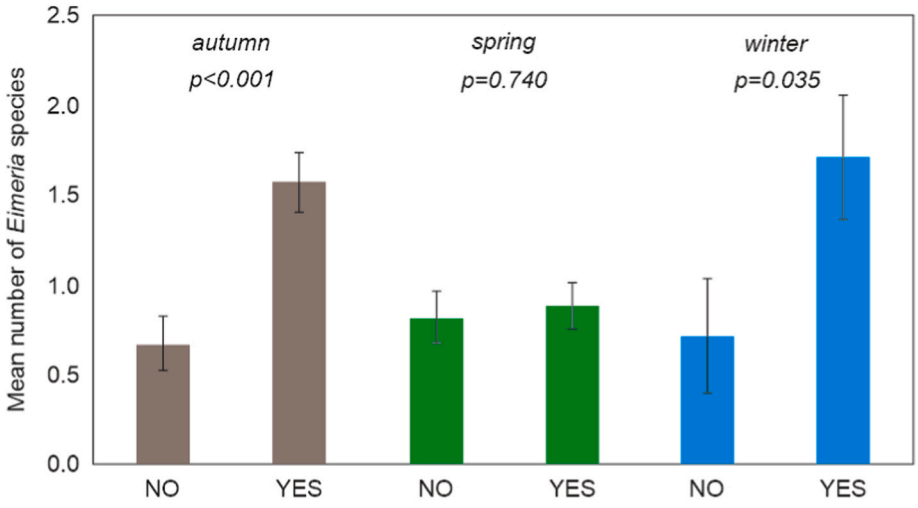

Fig. 2. Mean (±SE) number of *Eimeria* species in European bison without ungulate neighbors (N0) and kept near other ungulates (YES) for seasons, calculated in a generalized linear model. Differences were statistically significant in the pairwise comparison for autumn and winter (p values shown above the bars).

Vadlejch et al., 2017, Pyziel et al., 2018a). This also applies to coccidia, whose interspecies transmission between domestic and wild bovids has also been observed (Sayin, 1968; Penzhorn et al., 1994; Pyziel et al., 2014, 2020; Dubey, 2018). However, as shedding of parasite eggs and oocysts depends on many other environmental and individual factors (Larsson et al., 2006; Pyziel et al., 2011), the impact of other ungulates on the parasite species diversity in captive European bison should be thoroughly confirmed in the future.

Evaluation of deworming efficacy is a very important part of monitoring the health status of endangered animals. European bison have become extinct in the wild but their population has recovered from captive animals, thus highlighting the importance of monitoring the efficacy of anthelmintic treatment in captive populations. Since the results of our study indicate that anthelmintic treatments are unsatisfactory, it seems justified to continue study in this area. The potential sharing of parasite species between European bison and other ungulates should also be further investigated from the perspective of minimizing the risk of spreading drug-resistant parasite strains.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wanda Olech reports financial support was provided by Forest Fund Poland, contract no. OR.271.3.10.2017.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.06.005.

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