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Coccidia (Apicomplexa: Eimeriidae) of the lowland European bison *Bison bonasus bonasus* (L.)

Anna M. Pyziel[a,*], Michał Jóźwikowski[b], Aleksander W. Demiaszkiewicz[a]

[a] *W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland*
[b] *Institute of Mathematics, Polish Academy of Sciences, Śniadeckich 8, 00-956 Warsaw, Poland*

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ABSTRACT

Coprological studies conducted between 2007 and 2011 in free-roaming and captive European bison *Bison bonasus* (Linnaeus, 1758) from Poland revealed 11 species of *Eimeria* infecting the host, i.e., *Eimeria alabamensis*, *Eimeria auburnensis*, *Eimeria bovis*, *Eimeria brasiliensis*, *Eimeria bukidnonensis*, *Eimeria canadensis*, *Eimeria cylindrica*, *Eimeria ellipsoidalis*, *Eimeria pellita*, *Eimeria subspherica*, and *Eimeria zuernii*. The typical host for all isolated species is cattle. The most prevalent species was *E. bovis* (29.7%), while *E. brasiliensis* was the rarest (0.5%). Five of the species (*E. bovis*, *E. bukidnonensis*, *E. canadensis*, *E. ellipsoidalis*, *E. zuernii*) have been observed previously in bison by other authors, 3 species were noticed by us in bison previously (*E. alabamensis*, *E. cylindrica*, *E. pellita*), while for 3 species (*E. auburnensis*, *E. brasiliensis*, and *E. subspherica*) these are new host and locality records. Oocysts of two species (*E. brasiliensis*, *E. bukidnonensis*) were noted only in the feces of bison kept in captivity. Moreover, the prevalence of positive samples was higher in the group of captive animals (55.4%) in comparison with the free-roaming herds (29.5%); although, oocysts per gram (OPG), counted with the conventional McMaster technique, was comparable in both groups, reaching maximally 6550 and 6400 in free-roaming and captive individuals, respectively. Overall, 142 fecal samples from 424 samples examined were positive for *Eimeria* (prevalence = 33.5%). Age-related analysis revealed a higher percentage of *Eimeria* spp. positive samples and higher OPG values in bison under 1 year old as compared to older individuals (93.3% and 50–4050; 37.3% and 50–550, respectively). Additionally, greater eimerian species diversity was present among calves in comparison with older bison. In most cases single-species infections were observed (59.8%) with a predominance of *E. bovis* (85.9%). Multiple-species infections consisted of 2–7 species, usually including *E. bovis*. The observation was made that *E. bovis* infection appears conducive to the host acquiring more eimerian species. No symptoms of clinical coccidiosis occurred during the study.

1. Introduction

Bison bonasus (Linnaeus, 1758) (Artiodactyla, Bovidae) is the largest, terrestrial mammal of Europe, and the only wild free-living species of Bovidae in Poland. The Bialowieza Forest is currently inhabited by over 800 free-roaming lowland bison, derived from just 7 animals that were used in the process of restitution of the species after the First World War (Krasinska and Krasinski, 2007). Species of coccidia from the genus *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) infecting European bison have not been studied in detail to date, despite the fact that bison are one of

* Corresponding author. Tel.: +48 226978967.
E-mail address: anpyz@twarda.pan.pl (A.M. Pyziel).

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Table 1
Comparison of eimerian prevalence and OPGs (oocyst count per gram) between free-roaming and captive European bison.

Species	Free-roaming (n = 359)				Captive (n = 65)				Total (n = 424)			
	Prevalence		OPG		Prevalence		OPG		Prevalence		OPG	
	%	95% CI	X	Range	%	95% CI	X	Range	%	95% CI	X	Range
Eimeria alabamensis	2.2	(1.2–4.3)	531	50–2600	1.5	(0.4–8.2)	100	100	2.1	(1.2–3.9)	483	50–2600
E. auburnensis	1.9	(1.0–3.9)	743	50–4050	9.2	(4.5–19.0)	75	50–100	3.1	(1.9–5.1)	435	50–4050
E. bovis	27.6	(23.3–32.5)	233	50–6550	41.5	(30.9–54.4)	474	50–6150	29.7	(25.7–34.3)	284	50–6150
E. brasiliensis	–	–	–	–	3.1	(1.0–10.6)	50	50	0.5	(0.2–1.6)	50	50
E. bukidnonensis	–	–	–	–	4.6	(1.8–12.9)	183	50–450	0.7	(0.3–2.0)	183	50–450
E. canadensis	1.4	(0.7–3.2)	210	50–450	4.6	(1.8–12.9)	167	50–400	1.9	(1.0–3.6)	194	50–450
E. cylindrica	2.2	(1.2–4.3)	81	50–250	1.5	(0.4–8.2)	50	50	2.1	(1.2–3.9)	78	50–250
E. ellipsoidalis	3.9	(2.4–6.4)	128	50–650	6.2	(2.6–15.0)	75	50–100	4.5	(3.0–6.9)	113	50–650
E. pellita	0.6	(0.2–1.9)	175	150–200	7.7	(3.5–17.0)	80	50–150	1.6	(0.9–3.3)	107	50–200
E. subspherica	0.6	(0.2–1.9)	325	100–550	3.1	(1.0–10.6)	275	100–450	0.9	(0.4–2.3)	300	100–550
E. zuernii	7.8	(5.5–11.0)	177	50–2750	21.5	(13.6–33.4)	82	50–200	9.9	(7.5–13.1)	145	50–2750
Eimeria spp.	29.5	(25.2–34.5)	396	50–6550	55.4	(44.1–67.7)	471	50–6400	33.5	(29.3–38.2)	415	50–6550

Sample size (n), prevalence (% and 95% confidence interval), oocyst count per gram (average, X, and range).

the most thoroughly investigated species of wild ruminants in Poland. Five species of eimerians have been previously described from European bison, i.e., *Eimeria bovis*, *Eimeria bukidnonensis*, *Eimeria canadensis*, *Eimeria ellipsoidalis*, and *Eimeria zuernii* (Levine and Ivens, 1986; Golemansky, 2003; Demiaszkiewicz and Pyziel, 2009; Treboganova, 2010), while more recently we have recorded a further three species new for this host, i.e., *Eimeria alabamensis*, *Eimeria cylindrica*, and *Eimeria pellita* (Pyziel et al., 2011). The aim of the present study was to reveal the complete species composition of coccidia infecting European bison regarding free-roaming and captive herds on the basis of the morphological and development features of oocysts.

2. Materials and methods

Over a of 4-year period (2007–2011), we analyzed 424 fecal samples from lowland European bison *Bison bonasus bonasus* (L., 1758) to determine the morphotypes of coccidia that the species is a host for. Three hundred and fifty-nine samples were taken from free-roaming bison living in the forests of the north-east Poland (Bialowieza Primeval Forest, Knyszynska Primeval Forest, and Borecka Primeval Forest), including 293 samples taken from radio-collared animals and 66 samples taken directly from the rectum of euthanized individuals (51 samples from bison over 1 year old, and 15 samples from bison calves under 1 year old). An additional 65 samples were collected by bison-keepers from 7 captive herds situated throughout the country. Fresh fecal samples were placed in 50-ml tubes and investigated in the laboratory. Samples were examined by direct flotation in saturated sucrose solution using the McMaster quantitative method and sporulated as described in Pyziel and Demiaszkiewicz, (2013). Descriptions of oocyst morphology and the abbreviations for oocyst and sporocyst structures followed the guidelines of Duszynski and Wilber (1997). Oocyst characters included length (L), width (W), their range and ratio (L/W), micropyle (M), micropyle width (MW), micropyle cap (MC), micropyle cap width (MCW), micropyle cap depth (MCD), polar granule (PG), oocyst residuum (OR); sporocyst characters were length (L), width (W), their range and ratio (L/W), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR) and refractile bodies (RB). All dimensions were given in micrometers [mean ± SD (μm)].

All numerical computations were performed using Mathematica 4.0 software (Wolfram Research Inc., IL, USA). To calculate the 95% confidence intervals (95% CI) for the prevalence the exact (Clopper–Pearson) method for binomial probability distribution was used. The hypothesis that the infections of *E. bovis* and of other *Eimeria* species are independent was checked, with the standard binomial test (significance level 0.05), using n_{AB} – the number of bisons infected simultaneously by *E. bovis* and other *Eimeria* species A – as the test statistic. The increase of probability $\Delta p_{AB} = n_{AB}/(n_A \cdot n_B)$ is defined as the ratio of n_{AB}/n_B – the prevalence of species A in the population infected by *E. bovis* and n_A/n – the prevalence of species A in the total population. In other words, Δp_{AB} describes how much more likely the infection by species A is if a bison is already infected by *E. bovis*. The 95% CI for Δp_{AB} was calculated as above.

3. Results

We obtained and identified 11 morphotypes of oocysts from European bison (*E. alabamensis*, *Eimeria auburnensis*, *E. bovis*, *Eimeria brasiliensis*, *E. bukidnonensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. pellita*, *Eimeria subspherica*, and *E. zuernii*). Morphology of the oocysts we observed compared favorably with the descriptions of these species isolated from cattle (*Bos taurus*). Overall, 142 of 424 samples collected were positive (33.5%) and the OPG varied from 50 to 6550. The most prevalent species was *E. bovis* (29.7%), while *E. brasiliensis* was the rarest (0.5%) (Table 1). Comparison of free-roaming and captive bison revealed higher eimerian species diversity and a higher number of positive fecal samples in the captive herds (Table 1). The mean number of *Eimeria* species per positive fecal sample was 1.7 (range 1–7). Single-species shedding was predominant, reaching 59.8% (85 out of 142) and was caused mostly by *E. bovis* (85.9%). Other species involved in single-species infections were *E. zuernii* (8.2%), *E. pellita* (3.5%), *E. ellipsoidalis* (1.2%), and *E. brasiliensis* (1.2%). Mixed-species