

Patterns of parasite eggs, oocysts and larvae shedding by moose in the Biebrza marshland (NE Poland)

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ABSTRACT

The study analyses patterns of endoparasite eggs, oocysts and larvae shedding by moose from the relict population in the Biebrza marshland, NE Poland, which has grown to be one of the largest in Central Europe since the ban on hunting imposed in 2001. The analysis identified 10 species or groups of parasites among 230 faecal moose samples collected over 16 consequent months. The most prevalent were the eggs of Trichostrongylidae, *Trichuris* spp., *Nematodirella alcidis*, *Parafasciolopsis fasciolaemorpha* and the larvae of *Elaphostrongylus* sp. Four parasite species were more prevalent in males, indicating male-biased parasitism, and the studied moose population exhibited a female-skewed sex ratio. *Nematodirella alcidis* eggs and Protostrongylid larvae were more prevalent during winter, which indicated their resistance to harsh weather conditions. The prevalence of *Eimeria alces* and *Aonchotheca* sp. increased during the growing season, as did the number of eggs per gram of faeces (EPG) of *P. fasciolaemorpha*, possibly due to the availability of water sources. Higher mean monthly temperature was also found to have a positive effect on the excretion of Trichostrongylidae and *Moniezia* spp. eggs. In addition, the time of infection and the specificity of the parasite life cycle, being sensitive to certain climatic conditions, also appeared to have a strong influence on eggs, oocysts and larvae shedding in this non-harvested moose population.

1. Introduction

Parasites substantially influence key aspects of wildlife populations, including their reproduction and survival (Anderson, 1978); they also constitute an important part of bottom-up mechanisms, where several species compete for a common host resource (Lagrue and Poulin, 2015). The impact of parasites on the morbidity and mortality of ruminants has been clarified in a number of recent studies (Hoberg, 2005; Karamon et al., 2015; Kołodziej-Sobocińska et al., 2016b). Their spread in populations of wild terrestrial mammals and the resulting host-parasite interactions are influenced by numerous ecological factors, including season and population variables (Kołodziej-Sobocińska, 2019). High host population density may favour the risk of parasite transmission, and seasonal host migrations can impact parasite diversity, as well as load and epidemiology (Radwan et al., 2010; Altizer et al., 2011; Sugiura et al., 2018). Furthermore, parasite dynamics can

be affected by various biological factors, such as the age of the host, and its body condition and hormonal status (Kołodziej-Sobocińska, 2019). Understanding the host-parasite relationship and the impact of parasites on population dynamics has become an important issue in the management of wild animal species (Thompson et al., 2010).

The moose, *Alces alces* (Linnaeus, 1758), is a large mammalian herbivore (LMH) known to be widespread in Northern Europe, Siberia and North America (Hundertmark and Bowyer, 2004). The western border of its European range runs through Poland, which is also the southernmost area of its distribution (Ratkiewicz et al., 2011). Excessive exploitation of the species during the 1980s and 90s in Poland resulted in the population declining by over 70% (Gębczyńska and Raczyński, 2004). A subsequent ban on moose hunting, imposed in 2001 on the entire territory of Poland, has since resulted in significant population growth, with local moose densities exceeding 1.5 ind/km² in 2011 (Raczyński and Ratkiewicz, 2011). One of densest genetically-

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distinct populations of moose (Świśłocka et al., 2008, 2013) currently occupies the Biebrza marshland, which is one of the core areas of the species distribution in Poland (Ratkiewicz et al., 2011).

The habitat use by the moose in the Biebrza Valley is influenced by seasonal migration. In winter, moose gather in the pine forests at higher elevation, while during the growing season, the population is less clustered and animals utilize marshland and alder bog forests (Borowik et al., 2018). These conditions may have a significant impact on parasite dynamics, making the moose in the Biebrza marshlands an ideal model for studying parasite species distributions across non-harvested, autochthonous LMH host populations.

The aim of our study was to identify the internal parasites present in these moose populations and analyze their seasonal patterns of parasite shedding.

2. Materials and methods

2.1. Study area

The study was conducted in the Biebrza River Valley, located in north-eastern Poland (53° 24'25"N, 22° 47'43"E). The area is protected by the Biebrza National Park (59 233 ha) and encompasses the largest natural wetlands area of Central Europe. The marshland vegetation is dominated by sedge, sedge-moss and reed communities. The wet forests consist of black alder, downy birch and coniferous bog forests (Bartoszuk, 2005), which constitute the nutritional base for moose in the growing season. In the winter, the moose abandon the marsh boundaries and migrate in search for food to the elevated areas on the edge of the valley, which are covered by coniferous and mixed-coniferous forests (Sokołowski, 2006; Borowik et al., 2018).

The area of the Biebrza Valley is characterised by a combination of continental and sub-boreal climates with long winters and a short vegetation period. Winter lasts up to 117 days, with a typical maximum temperature below 0 °C, while summer ranges in length from 77 to 85 days with a mean daytime temperature of 15 °C. The mean temperature in the coldest month, February, ranges from −4.5 to −5.5 °C depending on the year. The warmest month is July, with the mean temperature ranging from +17.3 °C to +17.8 °C. Snow cover persists from 110 to 140 days. The mean annual precipitation is 550 mm (Banaszuk, 2004).

2.2. Faeces collection and analyses

A total of 230 aecal samples were collected from moose over 16 months (14.4 per month on average), from November 2012 to February 2014, in the area of the Biebrza marshland. Samples were collected in areas of GPS- locations of collared moose. Typically, several stool pellets were collected from a single heap in a given month, then cooled and stored in plastic tubes for microscopic analyses. Pseudo replication was avoided by employing both temporal and spatial stratification, i.e. no more than one or two faecal samples were collected from a given area in the same time.

Additionally, distinct individuals were identified from collected stool samples by genetic methods. Briefly, DNA was extracted from the faecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). Following this, each faecal sample was examined according to 11 microsatellite loci (Świśłocka et al., 2015), to identify multilocus genotypes of individual moose. In addition, the sex of the studied animals was determined by the analysis of a ~333-bp fragment of the SRY gene together with the microsatellite loci in set 2 (Świśłocka et al., 2015). Multiplex PCRs were performed with ~25 ng genomic DNA, 1.7 µL Qiagen multiplex PCR Master Mix (1 ×), 0.3 µL mix of primers, and 1 µL RNase-free water in a 5 µL reaction volume. The following thermocycling parameters were used: initial denaturation step at 95 °C for 15 min, followed by 35 cycles with denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 60 s, followed by

final elongation for 30 min at 60 °C. The multiplex PCRs were performed in a GeneAmp PCR System 9600 thermal cycler (Applied Biosystems). The multiplex PCR products were then separated by size by capillary electrophoresis (3130 Genetic Analyzer, Applied Biosystems) using the GeneScan 500 LIZ standard, and finally, the electromorphs were genotyped with GeneMapper 4.0 (Applied Biosystems).

Faecal samples, 3g each, were taken to determine the presence of helminths. Helminth eggs and other oocysts were identified by direct flotation in sucrose solution by centrifugation (Taylor et al., 2007), fluke eggs were identified by decantation in tap water according to Żarnowski and Josztowa (Ziomko and Cencek, 1995) and the larvae of lung nematodes were identified using the Baermann technique (Bowman, 2009).

The samples were investigated using a Jenaval light microscope (Carl Zeiss Jena, Germany) at 125x magnification (gastrointestinal helminth eggs and oocysts) and a stereoscopic microscope (PZO, Poland) at 40x magnification (fluke eggs, lung nematode larvae). The eggs, oocysts and larvae were identified to family, genus or species level on the basis of morphometrical features (Goliszewska and Demiaszkiewicz, 2007; Bowman, 2009; Lankester et al., 2011; Verocai et al., 2014; Filip et al., 2016).

Two parameters were used to present the levels of parasitic infections: prevalence, defined as the percentage of moose faeces in which parasite eggs/oocysts/larvae were detected at least once, and intensity, understood as the number of eggs/oocysts/larvae in 1 g of previously frozen and thawed faeces (EPG/OPG/LPG).

Both measures were analysed with respect to the season, climate conditions and sex of the moose to provide a more detailed description of the seasonal and sex-specific pattern of shedding eggs, oocysts and larvae by moose parasites. The year was divided into two seasons: winter (December–April) and the growing season (May–November). Weather data was obtained from the Polish Institute of Meteorology and Water Management - National Research Institute.

2.3. Statistical analyses

Categorical variables were expressed as absolute count (n) and percentage within a group. Comparisons between groups were performed using the Pearson's chi-square test, or by Fisher's exact test if the expected count in the contingency table was below 5. When more than two groups were compared, the chi-square test was used as an omnibus test: when it yielded a significant result, a *post hoc* analysis was performed according to Markowski and Markowski (2009). Briefly, the group with the greatest mean contribution to the chi-square total statistics was identified; this group was then removed from the contingency table and the chi-square test was performed again. The procedure was repeated until the chi-square test yielded an insignificant result. Ninety-five percent confidence intervals (95% CI) for prevalence were calculated according to the Wilson score method (Altman and Royston, 2000). Numerical variables were presented as median, interquartile range (IQR) and range, and were compared between groups using the Mann-Whitney *U* test (for comparison of two groups) or the Kruskal-Wallis H-test with Dunn's *post hoc* test (for comparison of more than two groups). Ninety-five percent confidence intervals (95% CI) for the median EPG/LPG were calculated as the range between the 2.5th and 97.5th percentile. Relationships between two numerical variables were investigated with the Spearman's rank correlation coefficient (r_s). All statistical tests were two-sided. The significance level (α) was set at 0.05. Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, USA).

3. Results

In total, 230 fresh faecal samples from 187 moose (identified genetically as 65 males and 122 females) were examined. After the elimination of multiple samples collected from the same moose

Table 1
Prevalence (95% confidence interval) of parasitic infections in moose in the Biebrza marshland with regard to moose males and females.

Parasite	General (n = 187)		Males (n = 65)		Females (n = 122)		Chi-squared test (p)
	n of positive samples	Prevalence (95%CI)	n of positive samples	Prevalence (95%CI)	n of positive samples	Prevalence (95%CI)	
Trichostrongylidae	180	96.3 (92.5–98.2)	65	100 (94.4–100)	115	94.3 (88.6–97.2)	0.098
<i>Nematodirella alcidis</i>	91	48.7 (41.6–55.8)	37	56.9 (44.8–68.2)	54	44.3 (35.8–53.1)	0.099
<i>Aonchotheca</i> sp.	11	5.9 (3.3–10.2)	2	3.1 (0.8–10.5)	9	7.4 (3.9–13.4)	0.388
<i>Trichuris</i> spp.	100	53.5 (46.3–60.5)	43	66.1 (54.0–76.5)	57	46.7 (38.1–55.5)	0.011
<i>Elaphostrongylus</i> sp.	31	16.6 (11.9–22.6)	15	23.1 (14.5–34.6)	16	13.1 (8.2–20.2)	0.081
<i>Varestrongylus</i> sp.	11	5.9 (3.3–10.2)	7	10.8 (9.7–27.8)	4	3.3 (1.3–8.1)	0.038
<i>Dictyocaulus</i> sp.	8	4.3 (2.2–8.2)	6	9.2 (4.3–18.7)	2	1.6 (0.5–5.8)	0.022
<i>Moniezia</i> spp.	10	5.3 (2.9–9.6)	2	3.1 (0.8–10.5)	8	6.6 (3.4–12.4)	0.505
<i>Parafasciolopsis fasciolaemorpha</i>	168	89.8 (84.7–93.4)	62	95.4 (87.3–98.4)	106	86.9 (79.8–91.8)	0.067
<i>Eimeria alces</i>	8	4.3 (2.2–8.2)	6	9.2 (4.3–18.7)	2	1.6 (0.5–5.8)	0.022

individuals during the same season, determined by their multilocus microsatellite genotypes, 99 samples (49.8%) remained from the growing season and 100 samples (50.2%) from the winter season. After removal of samples acquired from the same moose in different months, the genetically identified sex ratio in the studied, non-harvested relic moose population was found to be female-skewed, equalling 1 male: 1.69 female.

The faeces were found to contain eggs, oocysts and larvae from 10 species or groups of parasites (Table 1): gastrointestinal nematodes from the family Trichostrongylidae, *Nematodirella alcidis*, nematodes from the genera *Aonchotheca* and *Trichuris*, *Eimeria alces*, tapeworms from the genus *Moniezia*, fluke *Parafasciolopsis fasciolaemorpha*, as well as lung nematodes from the family Dictyocaulidae (*Dictyocaulus* sp.), and Protostrongylidae (*Elaphostrongylus* sp. and *Varestrongylus* sp.).

The most prevalent were the Trichostrongylidae eggs, found in 96.3% of examined samples, followed by *P. fasciolaemorpha* eggs (89.8%), *Trichuris* spp. nematodes (53.5%) *N. alcidis* eggs (48.7%) and larvae from the genus *Elaphostrongylus* (16.6%) (Table 1). The prevalence of the other parasites did not exceed 6% (Table 1). Median EPG values were low: the highest values were found for *Moniezia* spp. (30 EPG) and the Trichostrongylidae family (14 EPG). Four parasites were found to be present significantly more often in males than females: *Trichuris* spp. ($p = 0.011$), *Varestrongylus* sp. ($p = 0.038$), *Dictyocaulus* sp. ($p = 0.022$) and *Eimeria alces* ($p = 0.022$) (Table 1).

The prevalence and intensity of parasitic infections differed depending on the season and climate conditions. The prevalence of *N. alcidis* was significantly lower during the growing season (Table 2), especially from May to September (Table S1; Fig.S1 in the Supplementary Material), and decreased together with higher mean monthly temperature (Fig. 1).

The Protostrongylid nematodes *Elaphostrongylus* sp. and *Varestrongylus* sp. were less prevalent during the growing season (Table 2). Larvae excretion by *Elaphostrongylus* sp. peaked twice – once

in winter and second time in spring (Fig. 2) and was positively correlated with the presence of snow cover (Fig. 3).

The prevalence of *Eimeria alces* and *Aonchotheca* sp. was significantly greater during the growing season (Table 2), especially in September and October (Table S1 in the Supplementary Material).

A higher mean monthly temperature was associated with the greater prevalence and EPG of *Moniezia* spp. as well as higher EPG for Trichostrongylidae family and *P. fasciolaemorpha* (Figs. 1–2). The level of eggs excretion of *P. fasciolaemorpha* was higher in the growing season, when mean monthly temperature was increasing, and lower during months with snow cover (Figs. 2–3), especially in January and February (Fig.S2 in the Supplementary Material).

4. Discussion

We detected a minimum 10 species of parasites in the faeces from moose of the Biebrza marshes, NE Poland. A similar profile was previously obtained for managed moose populations in Scandinavia (Milner et al., 2013; Davidson et al., 2015). Interestingly, high numbers of *P. fasciolaemorpha* eggs were also detected in the study population; these were not observed in the Scandinavian moose, possibly because Northern Europe is outside the range of the fluke (Filip and Demiaszkiewicz, 2016). The most common parasites were generally more widely prevalent in the present study than in Milner et al. (2013) and Davidson et al. (2015). This does not suggest density-dependence, as moose densities are similar in the Biebrza valley and Scandinavia (1.5 ind./km², Ratkiewicz et al., 2011, 1,3 ind./km², Milner et al., 2012). Despite their high prevalence, the intensity of parasitic infections in our study was very low (Table 3) and did not entail any clinical signs of the disease. Although faecal egg count sometimes reflects true parasite burdens (Irvine et al., 2000; Ball et al., 2001), it depends on many factors, such as the immune status of the host and fecundity of the parasite, and should be interpreted with caution (Davidson et al.,

Table 2
Prevalence (95% confidence interval) of parasitic infections in moose during the growing and winter seasons.

Parasite	Growing season (n = 99)		Winter season (n = 100)		Chi-squared test (p)
	n of positive samples	Prevalence (95%CI)	n of positive samples	Prevalence (95%CI)	
Trichostrongylidae	93	93.9 (87.4–97.2)	98	98.0 (93.0–99.4)	0.145
<i>Nematodirella alcidis</i>	27	27.3 (19.5–36.8)	67	67.0 (57.3–75.4)	< 0.001*
<i>Aonchotheca</i> sp.	10	10.1 (5.6–17.6)	1	1.0 (0.2–5.4)	0.005*
<i>Trichuris</i> spp.	48	48.5 (38.9–58.2)	56	56.0 (46.2–65.3)	0.289
<i>Elaphostrongylus</i> sp.	10	10.1 (5.6–17.6)	22	22.0 (15.0–31.1)	0.022*
<i>Varestrongylus</i> sp.	2	2.0 (0.6–7.1)	9	9.0 (4.8–16.2)	0.031*
<i>Dictyocaulus</i> sp.	4	4.0 (1.6–9.9)	4	4.0 (1.6–9.8)	0.988
<i>Moniezia</i> spp.	6	6.1 (2.8–12.6)	3	3.0 (1.0–8.5)	0.299
<i>Parafasciolopsis fasciolaemorpha</i>	91	91.9 (84.9–95.8)	87	87.0 (79.0–92.2)	0.259
<i>Eimeria alces</i>	7	7.1 (3.5–13.9)	1	0.2 (0.2–5.4)	0.029*

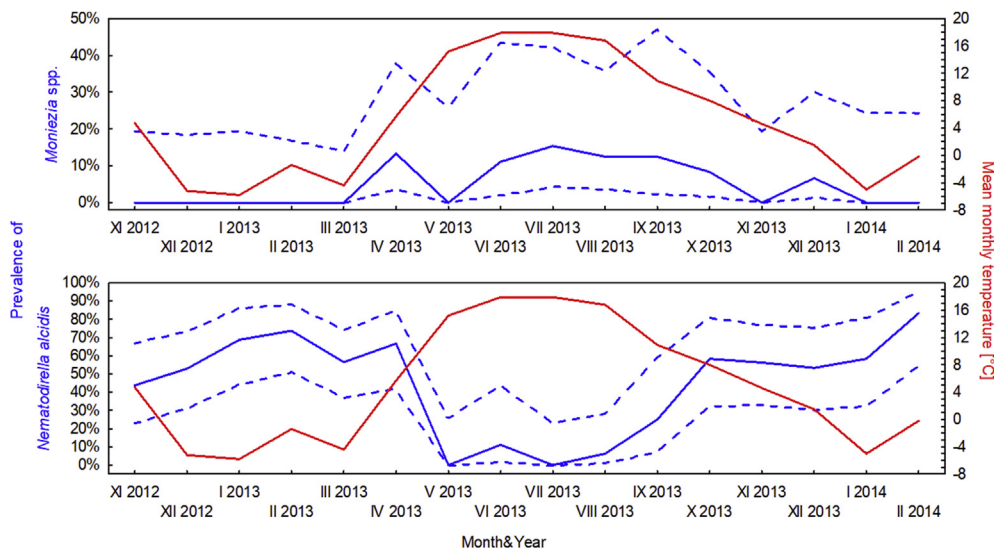


Fig. 1. The relationship between the prevalence of *Nematodirella alcidis* and *Moniezia* spp. eggs and mean monthly temperature (red continuous line). Blue continuous line shows the prevalence and broken lines indicate its 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

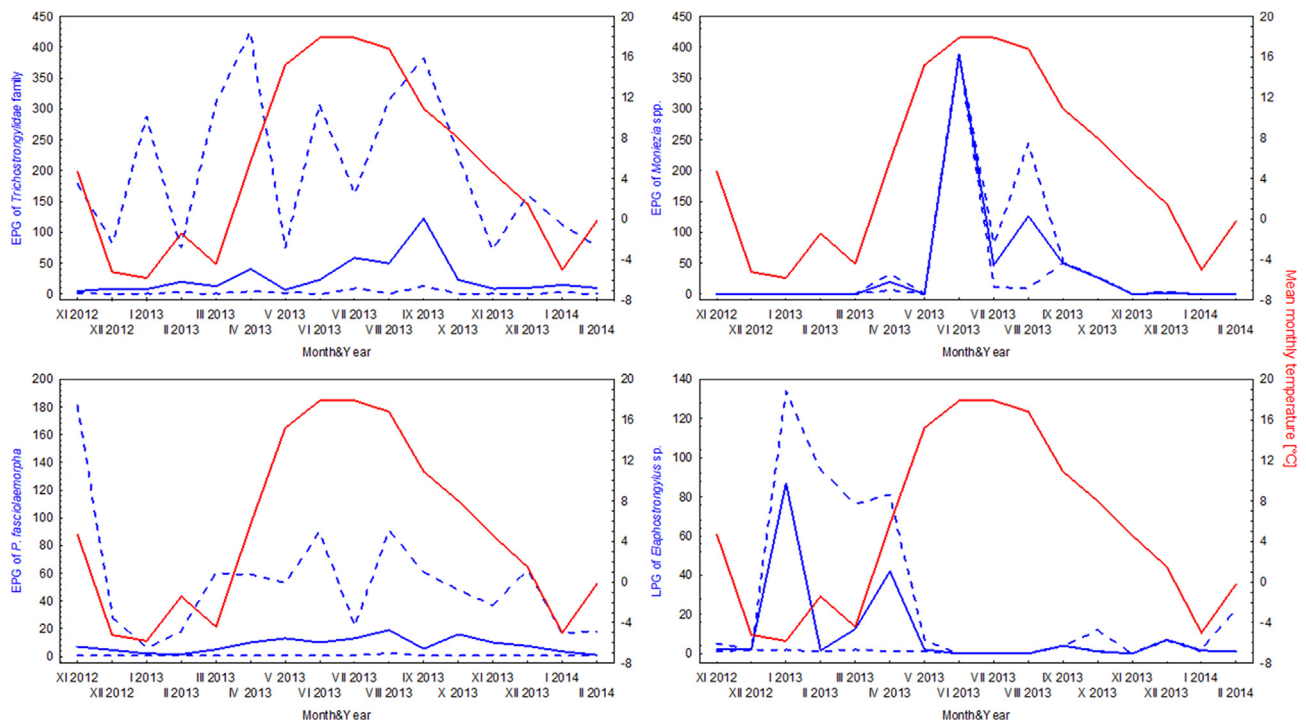


Fig. 2. The relationship between the EPG of Trichostrongylidae family members, *Moniezia* spp., *Parafasciolopsis fasciolaemorpha*, the LPG of *Elaphostrongylus* sp. and mean monthly temperature (red continuous line). Blue continuous line shows the median EPG/LPG and broken lines indicate its 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2015).

The prevalence of *Trichostrongylus* spp., *Varestrongylus* sp., *Dictyocaulus* sp. and *Eimeria alces* was significantly higher in males than females. Grear et al. (2012) proposes that male moose are more susceptible to infection than females, despite similar levels of exposure to parasites, due to the immunosuppressive effect of testosterone and increased vulnerability to infection. Other authors have noted that males provide greater numbers of transmission events, due to higher exposure to infective forms of parasites associated with *inter alia* increased food intake (Halvorsen, 1986).

The winter of 2012/2013 in the Biebrza marshland was exceptionally long and harsh with the snow cover persisting until mid-April. During this season, high mortality rate was observed among male moose (Ratkiewicz et al., pers. commun.). Unfortunately, these males

were not studied with respect to their parasite prevalence and intensity. Interestingly, Moore and Wilson (2002) report a positive correlation, across taxa, between male-biased mortality and male-biased parasitism. Hence, it cannot be ruled out that male-biased parasitism, being a source of male-biased mortality, could have resulted in female-skewed sex ratio in this non-harvested moose population. Alternatively, this may be caused by some unnoticed human impact on the male moose in the studied population, of perhaps the negative impact of *Trichostrongylus* spp. on health status, as the parasite is known to contribute to Wasting Disease Syndrome in moose (Clausen et al., 2002). On the other hand, increased parasitism has been reported in females during some seasons due to the immunosuppressive effect of pregnancy and hormonal status during the parturition and lactation periods (Lloyd, 1983); however it was not observed during this study.

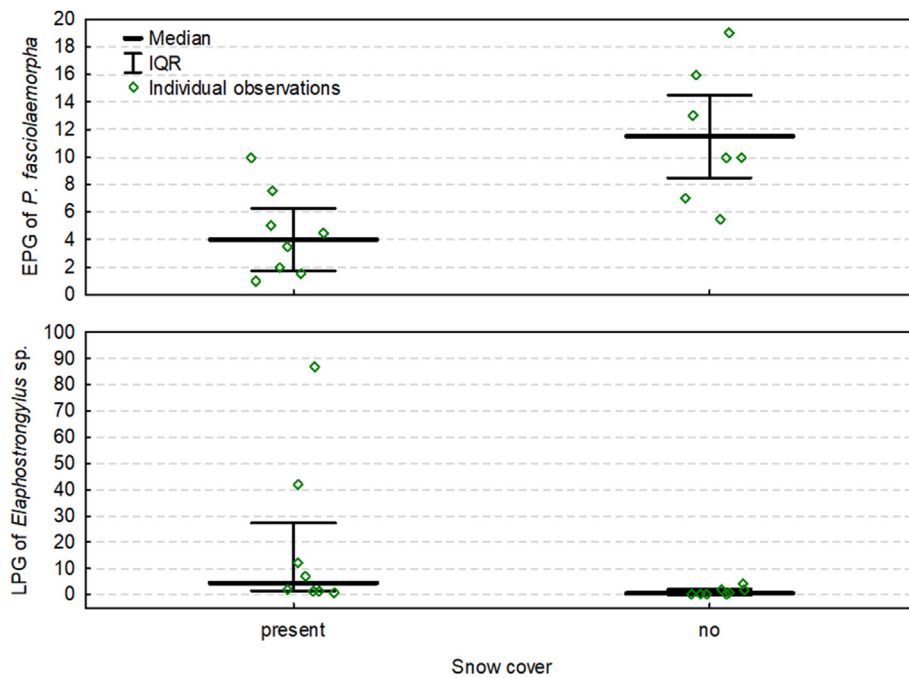


Fig. 3. The relationship between the median EPG of *Parafasciolopsis fasciolaemorpha*, the median LPG of *Elaphostrongylus* sp. and the presence of snow cover.

The host-parasite relationship can be affected by a variety of abiotic and biotic factors, such as climate, population density and seasonal migration: a strategy observed in many ungulates in the Northern hemisphere (Altizer et al., 2006; Borowik et al., 2018; Kołodziej-Sobocińska, 2019). At the end of the growing season, moose typically move from wetland areas to coniferous forests, where they stay for winter (Borowik et al., submitted 2018). The European bison, *Bison bonasus* (Linnaeus, 1758), has been found to exhibit a higher prevalence of parasitic infection in winter season, and this has been associated with more intensive clustering of animals, which favours parasite transmission (Kołodziej-Sobocińska et al., 2016a); however, no clear tendency to produce higher parasite egg numbers was observed during the winter season in the present study.

Among gastrointestinal nematodes, only the prevalence of *N. alcidis* was found to be higher in the winter season; however, this does not necessarily correspond with high parasite burdens (Fruetel and Lankester, 1988). Although most moose are infected with numerous *N. alcidis* larvae, most of these are arrested in development and comparatively few are mature nematodes that produce eggs. As these eggs are resistant to freezing but susceptible to high temperatures (Fruetel

and Lankester, 1988; Kutz et al., 2012), the increased excretion of *N. alcidis* eggs observed during harsh winter conditions in the present study (Figs. 1 and 2) might be only partially related to environmental factors.

The larvae of *Elaphostrongylus* sp. were more intensively excreted during months when snow cover was present, with a peak in February (Figs. 2–3). Other nematodes from the Protostrongylidae demonstrate similar seasonal dynamics, but with larval output peaking in winter/early spring (Halvorsen et al., 1985; Demiaszkiewicz, 1987; Jenkins et al., 2006). Jenkins et al. (2006) report that up to 60% of the first-stage larvae of *Parelaphostrongylus odocoilei*, *P. tenuis* and *Protostrongylus stilesi* are able to survive over winter to infect intermediate hosts at snowmelt. However, the survival of first-stage Protostrongylidae larvae has previously been found to decrease with increasing temperature (Rose, 1957; Shostak and Samuel, 1984; Lorentzen and Halvorsen, 1986; Jenkins et al., 2006): a characteristic also observed in our present study. Protostrongylids infect mostly young animals, and the existence of parasites in yearlings seems to confer immunological protection against new infections in subsequent years. This immunologically-determined threshold number of adult worms is one possible cause of the

Table 3

Intensity of parasitic infections in moose during the growing and winter seasons.

Parasite	n of positive samples	Growing season	n of positive samples	Winter season	p-value ^a
		Median, IQR (range)		Median, IQR (range)	
Trichostrongylidae	93	16, 5–81 (1–382)	98	13, 6–42 (1–426)	0.471
<i>Nematodirella alcidis</i>	27	1, 1–4 (1–5)	67	2, 1–5 (1–21)	0.161
<i>Aonchotheca</i> sp.	10	0, 0–0 (0–2)	1	–	^b
<i>Trichuris</i> spp.	48	7, 2–34 (1–454)	56	13, 2–61 (1–386)	0.289
<i>Elaphostrongylus</i> sp.	10	2, 1–5 (1–12)	22	4, 1–64 (1–134)	0.171
<i>Varestrongylus</i> sp.	2	1, 4	9	2, 1–4 (1–6)	^b
<i>Dictyocaulus</i> sp.	4	2 (1–5)	4	2 (1–4)	> 0.999
<i>Moniezia</i> spp.	6	66, 27–244 (9–388)	3	7 (2–33)	0.093
<i>Parafasciolopsis fasciolaemorpha</i>	91	12, 2–22 (1–181)	87	5, 2–10 (1–62)	0.001*
<i>Eimeria alces</i>	7	1, 1–28 (1–56)	1	1	^b

IQR-interquartile range.

^a Mann-Whitney U test.

^b Not tested.

poor correlation between Protostrongylid transmission and host density (Ball et al., 2001).

Our findings indicate that the prevalence of most common parasites in the present study did not increase during the growing season, except for the less prevalent nematodes, *Aonchotheca* sp., and coccidia, *Eimeria alces*. Both groups of parasites were excreted more often in September and October, i.e. during the rutting period when male moose move across large distances in search for females. Pyziel and Demiaszkiewicz (2013) report that watering places favour the transmission of coccidia between moose in the Białowieża Primeval Forest and suggest that this might be the case in the Biebrza marshland; however, the impact of reduced immune response during moose breeding season should not be excluded.

Higher EPG values in the growing season were observed for the fluke *P. fasciolaemorpha*, the second most prevalent parasite observed during the study. Egg shedding increased with mean monthly temperature, and decreased with the presence of snow cover (Figs. 2–3). The presence of the fluke in the environment is closely associated with the presence of water snails as intermediate hosts (Lachowicz, 1988), and as such, its life cycle tends to be strictly reliant on temperature and the availability of water sources; these factors may account for the higher level of egg shedding observed during the growing season.

A similar tendency, i.e. producing higher numbers of eggs at higher mean monthly temperatures, was observed in the most prevalent parasites from the Trichostrongylidae family, as well as in the less prevalent *Moniezia* spp. (Fig. 2). This may result from the spring rise phenomenon, the characteristics of the gastrointestinal nematodes, or it may be associated with the life cycle of parasites such as *Moniezia* spp., which need higher temperatures for further development (Narsapur and Prokopic, 1979).

Our findings do not allow it to be stated unequivocally whether the dynamics of parasitic infections were affected by high density of non-harvested moose population. Although the moose populations in Poland are undergoing high demographic expansion, those animals do not live in herds and the average cluster size for this species in Biebrza marshland is only two individuals (Ratkiewicz et al., pers. commun.). Annual shedding of eggs, oocysts and larvae of parasites depends on many intra- and extra-host factors and might be reduced by the sex, age and immune status of the host, as well as by the long lifespan of nematodes and the specificity of their life cycles, which may be sensitive to certain climatic conditions (Halvorsen et al., 1985; Ball et al., 2001; Jenkins et al., 2006).

Nevertheless, our results provide a greater insight into the host-parasite interactions occurring in a non-harvested moose population subjected to natural factors, including climatic variability and phenology, and could be regarded as a reference for other studies performed in managed moose populations. Faecal analysis is an easily available, cost-effective and non-invasive method of estimating the type and level of parasitic infections in non-harvested and protected animal species, and as such, faecal sample analysis should become part of the regular monitoring of non-harvested moose populations. In the future, genetic identification of parasites, using diagnostic PCR, should be performed to assess the level of parasite transmission more precisely and to verify microscopic studies.

An important limitation of this study is its relatively short study period, being only 16 months in length. This is arguably too short to fully understand and confirm the processes and factors affecting excretion of eggs, oocysts and larvae of parasites. Therefore, the observed patterns should be confirmed in subsequent years.

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Declaration of competing interest

None.

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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.2020.02.007>.

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