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INTESTINAL MACRO- AND MICROPARASITES OF WOLVES (*CANIS LUPUS* L.) FROM NORTH-EASTERN POLAND RECOVERED BY COPROLOGICAL STUDY

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Abstract: Wolf scats collected during ecological studies in Mazury lake district in NE Poland were analysed for intestinal micro- and macroparasites. Five nematode species were identified: *Ancylostoma caninum* (Ercolani, 1859), *Uncinaria stenocephala* (Railliet, 1884), *Trichuris vulpis* (Froelich, 1789), *Toxocara canis* (Werner, 1782) and *Toxascaris leonina* (von Linstow, 1902). Among cestode species there were identified infections with *Dipylidium caninum* (Linnaeus, 1785). The overall helminth prevalence was 63.5% and average intensity was 15.4 ± 8.0 eggs /1g of sample. The most prevalent parasite was *T. vulpis* (38.5%) and the most abundant infections were by *T. canis.* Almost 55% of samples (28/51) were positive for *C. parvum* oocysts and 46.7% (14/30) for *Giardia* spp. cysts. The pack factor affected the distribution of some of macro- and microparasites. The identified parasite fauna of wolves in Mazury lake district consists of several micro- and macroparasites of interest for public health.

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INTRODUCTION

Since 1998, when wolves became a protected species in Poland, they have spread and are now settled permanently in the east, north-east and south part of the country, including Mazury lake district [17]. A pack home range varies from 100 to 359 km^2 with an average of 230 km^2 [28, 31]. Wolf territories often cover managed forests and farmlands in human neighbourhoods resulting with direct or indirect contact of wolves with domestic animals and humans. Wolves are natural hosts for a wide range of intestinal parasites and some of these parasites can also infect humans and domestic animals [32]. In the life cycles of several helminth species, such as nematode *Toxocara canis* or cestodes like *Echinococcus* spp. or *Dipylidium caninum*, humans are involved as paratenic hosts with all its disadvantages. Migrating *T. canis* larvae can cause severe damages while incysted in the spinal cord, brain or eye [1]. Similarly, the growing *Echinococcus* cyst may be a reason for severe liver, brain, lung or bone damage, or even cause the death of a human host. *D. caninum*, which localizes in the small intestine, is a common parasite of dogs and can also develop in humans after accidental consumption of the intermediate host, a flea. Some helminth eggs, e.g. *T. canis*, are environmentally resistant, and remain infective for a long time [1]. Because wolves mark their territories by scats and urine [42] and sporadically prey on livestock [17], the possibility of parasite transmission between wolves and humans or domestic animals may become a risk factor for public health.

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Wolves are known as a reservoir hosts for some microparasites. Antibodies against *Toxoplasma gondii* were found in 9% of wolf blood samples examined in Alaska [41]. However, there is no report on natural infection with opportunistic human pathogens such as *Cryptosporidium parvum* or *Giardia duodenalis* in wolves. Both these protozoan intestinal parasites are the reason for chronic and severe diarrhea in immunocompromised individuals, and both have a wide range of animal reservoir hosts [13, 26, 27]. In zoonotic transmission with these microparasites, the crucial role is played by environmentally-resistant dispersal and infective stages (cysts or oocysts) which are excreted in large quantities with faeces of the infected host. The infection occurs not only by the direct faecal-oral route, but also by an indirect route, where the contaminated water or food serves as a source of infective stages [13]. The high risk of surface water contamination and waterborne infections in humans and animals occurs in the natural environment inhabited by the wide range of parasite natural hosts. In Poland, natural *C. parvum* infections were detected in livestock [7, 19] and in wild rodents [3, 5], but the role of other mammals, including carnivores, is still unknown.

The aim of this paper was to assess the role of wolves in contamination of the human environment with infective stages of micro- and macroparasites, based on the examination of wolf fecal samples collected during ecological study in NE Poland.

MATERIALS AND METHODS

Materials. Wolf scats were collected during field studies on wolf ecology in Puszcza Piska and Napiwodzko-Ramuckie forests (20°25'–21°51'E and 53°18'–53°47'N) in NE Poland (Kloch and Jędrzejewski, unpublished). In the present work we analysed feces collected during winter months in 2001/2002. Estimated home ranges of packs and number of wolves in packs are shown on Figure 1, based on ecological studies in the region (Kloch and Jędrzejewski, unpublished).

Wolf feces were identified due to their morphology, size and shape and the presence of wolf trails or footprints on the vicinity. Wolf scats are considerably bigger and have a different shape than those of other wild carnivores living in Poland - raccoon dog or red fox, and because of the presence of prey bones and hairs they can be distinguished from dog scats [36]. Only lynx scats could be mistaken, but this species does not occur in the study area [17]. A total of 57 wolf fecal samples was collected. Feces were frozen at -20°C prior to analysis.

Methods. Coprological survey for helminths was carried out using 2 methods: Fulleborn flotation technique (average sample weight 0.93 g ± 0.87) and decantation technique (average sample weight 0.74 g ± 0.34). Flotation was performed with test tubes filled to the top with fecal solution and saturated NaCl solution. A cover glass was placed on top for 20 min., then removed, placed on a microscope slide and examined under 160 × magnification. For decantation techniques, fecal samples were homogenized in 500 ml of distilled water. After 2 hours the water was gently removed and the sediment poured again with water; the procedure was repeated 3 times. Six volumes of 0.5 ml of condensed sediment were examined under 160 × magnification.

Helminth eggs were identified using the key [37]. Since both methods did not allow to distinguish between *Uncinaria stenocepaha* and *Ancylostoma caninum*, 2 nematodes with a similar life cycle were treated as the *Ancylostoma*/*Uncinaria* group and counted together in the analysis.

Detection of intestinal protozoa was carried out using 2 methods: modified Ziehl-Neelsen staining of fecal smears [16] and immunofluorescent assay MeriFluor *Cryptosporidium*/*Giardia* (Meridian Diagnostics, Cincinnati, Ohio, USA) on samples condensed by the Sheather flotation technique, as described previously [3, 14].

Statistical analysis. Prevalence data (percentage of samples infected) were analysed by maximum likelihood techniques, based on log-linear analysis of contingency tables using the software package Statgraphics Version 7 [3, 4]. Beginning with the most complex model, involving all possible main effects and interactions, those combinations which did not contribute significantly to explaining variation in the data, were eliminated in a stepwise fashion beginning with the highest–level interaction. A minimum sufficient model was then obtained, for which the likelihood ratio of χ^2 was not significant, indicating that the model was sufficient in explaining the data. The full factorial model initially comprised 3 factors at maximum (pack, prevalence of nematodes, prevalence of cestodes) and the infected/uninfected factor.

Summary figures for parasite abundance are expressed as means of LOG10(x+1) ± SE transformed data (corresponding to geometric means). Where relevant, the latter are also given as back–transformed values. These means reflect the abundance of infection as defined previously [9, 22] and include all subjects within the specified group, infected and not infected, for which relevant data were available. The degree of aggregation in the data was calculated by the Index of Dispersion (*I*), variance to mean ratio, where values >1 indicate overdispersed data.

Frequency distributions of individual helminth species were tested for goodness of fit to the normal distribution, the positive binomial distribution (assumption of the null model is a regular distribution), the Poisson distribution (assumption of the null model is a random distribution), and the negative binomial model (assumption of the null model is an aggregated distribution). All distributions were tested for goodness of fit by χ^2 as described by [12].

Parasite abundance was analyzed by GLIM, statistical system for generalized linear interactive modeling; GLIM 4, PC version, Royal Statistical Society 1993; [11, 39], as

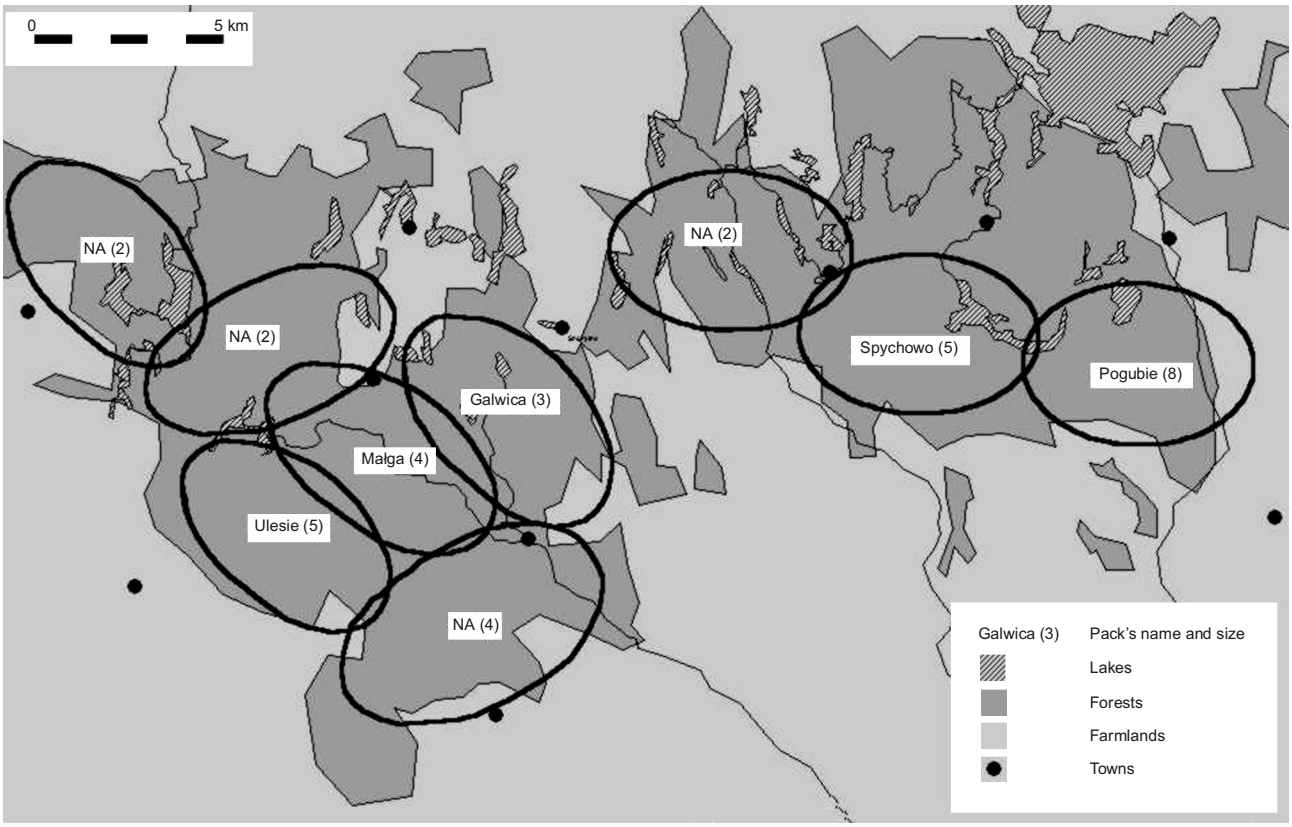

Figure 1. Home ranges estimations for wolf packs and number of individuals in packs.

described previously [3, 4, 8], using models with normal errors after normalization of the data by LOG10 (x+1) transformation. Pack (3 levels), prevalence of nematodes (2 levels: infected or uninfected), prevalence of cestodes (2 levels: infected or uninfected) were entered as factors. We began in all cases with the full factorial models, including all main effects and interactions, and then progressively simplifing them by deletion of terms, beginning with the highest order interactions, and progressing to the main effects. Three-way interaction was first deleted to register the change in deviance. The first 2-way interactions was then removed, and then reinstated in turn until all had been evaluated. The procedure was repeated for all 2-way interactions and for the main effects. For models with normal errors the change in deviance is divided by the scale parameter and the result divided by the change in degrees of freedom (df) following each deletion, to give a variance ratio, *F*. Finally, minimum sufficient models were fitted, entering only the significant terms.

RESULTS

Wolves *Canis lupus*. A total of 57 wolf scats were collected during winter 2001/2002. The spatial analysis of tracking data and samples distribution revealed the presence of 25 wolves grouped in 5 packs (Kloch and Jędrzejewski, unpublished). Since available data did not allow the reliable assessment of pack territory, we used estimated pack ranges based on radiotracking data from other lowland forests in Poland (Fig. 1) [28]. Pack territories consisted mainly of managed mixed and coniferous forests, meadows, wastelands and villages.

Measures of component community structure

Total helminth species richness and component species. In total, 6 species of helminths were recorded, 4 nematodes (with *Uncinaria* sp. and *Ancylostoma* sp. treated together) and at least 2 species of cestodes (*Dipylidium caninum* and unidentified ones) (Tab. 1). 63.5% of samples carried at least 1 of these species. The *Trichuris vulpis* infection was the most prevalent (38.5%), followed by the *Uncinaria*/*Ancylostoma* group (31%) and *Toxocara canis* (13.5%). All the other species were only sporadically represented, with overall prevalence not exceeding 10%. Therefore, no helminth species can be considered a core species (prevalence >50%) and 3 species (*T. vulpis*, *T. canis* and *Uncinaria*/*Ancylostoma* complex) are the component species (>10%) in this population of wolves, although the prevalence of all tapeworms was 13.5%. However, prevalence of some species varied between packs and in relation to different detection methods.

Total species richness, dominant species, diversity and similarity by pack. The total number of helminth species recorded in our study site in each of the 5 packs is given in Table 2. Most species were recorded in samples collected from Galwica pack territory from where the

Table 1. Prevalence (% infected) and abundance (geometric means) of parasite taxa.

Taxon	Species	Total			By pack (% infected)				
		number of samples tested	% infected	geometric mean ± SE	Galwica n=24	Malga n=17	Pogubie n=3	Spychowo n=2	Ulesie n=6
Helminths									
Nematodes	*Trichuris vulpis*	52	38.5	1.59 ± 1.22	41.7	20.4	33.3	0	66.6
	Uncinaria/Ancylostoma	52	30.8	2.24 ± 1.22	33.3	17.6	33.3	50	50
	Toxocara canis	52	13.5	2.04 ± 1.23	12.5	5.9	0	50	33.3
	Toxascaris leonina	52	3.8	1.26 ± 1.05	4.2	5.9	0	0	0
All nematodes		52	59.6	4.22 ± 1.34	66.7	47.1	33.3	50	83.3
Cestodes	*Dipylidium caninum*	52	3.8	1.05 ± 1.04	4.2	0	0	0	16.7
	unidentified tapeworms	52	9.6	1.44 ± 1.08	12.5	0	33.3	50	0
All cestodes		52	13.5	1.51 ± 1.09	16.7	0	33.3	50	16.7
All helminths		52	63.5	4.36 ± 1.34	75.0	47.1	33.3	50	83.3
Intestinal protozoa									
	Cryptosporidium parvum	51	54.9	15.01 ± 2.37	41.7	63.2	nd	0	100
	Giardia spp.	33	45.5	50.25 ± 2.38	35.7	50	nd	nd	75
All intestinal protozoa		51	64.7	84.93 ± 2.39	50	78.9	nd	0	100

nd - not detected,

Table 2. Comparison of helminth component community structure by pack.

		Galwica	Malga	Pogubie	Spychowo	Ulesie
Total number of helminth species identified		6	4	3	3	4
Dominant species		*T. vulpis*	*T. vulpis*	none*	none*	*T. vulpis*
Simpson's index						
Shared species	Galwica	xxx	4	3	2	4
	Malga	4	xxx	2	2	3
	Pogubie	3	2	xxx	2	2
	Spychowo	2	2	2	xxx	2
	Ulesie	4	3	2	2	xxx

* too small sample size

majority of samples was derived. In terms of similarity, (see shared species; Tab. 2), Galwica, Malga and Ulesie packs were close to one another, sharing 4 helminth species, and major contributors to this difference was the sample size. In all 3 cases, *T. vulpis* was the most prevalent species at the component community level.

Both species of intestinal protozoa, *C. parvum* and *Giardia* spp. were identified in the samples from 3 packs and both were absent in 2 samples from Spychowo pack (Tab. 1).

Measures of infracommunity structure

Mean species richness. The overall mean number of helminth species per sample (all samples combined) was 1.00 ± 0.14 (variance to mean ratio = 1.0588). Mean species richness did not vary significantly between packs; however, the highest mean species richness was observed in samples from Ulesie pack territory and the lowest in the Malga pack (Tab. 3).

Measures of infracommunity diversity. The maximum number of helminth species per sample ranged from 2 in samples from Malga pack to 4 in sample from Ulesie pack (Tab. 3). The geometric mean number of helminth ova per sample did not vary markedly between packs (Galwica: 3.49 ± 1.33; Malga: 2.36 ± 1.40; Pogubie: 2.08 ± 2.24; Spychowo: 19.67 ± 2.68; Ulesie: 4.65 ± 1.77). Distribution of total worm burdens (total number of helminth ova/g of sample) differed significantly from all tested distributions (positive and negative binomial, Poisson and normal distributions; $p<0.001$); however, the index of dispersion, I=225.2, indicated overdispersed data.

Species density distributions. In 3 of the 5 packs, the majority of samples contained ova of 0 or 1 helminth species (Galwica 75%, Malga 88.2%, Ulesie 66.7%), but in the Pogubie and Spychowo packs, in a single positive sample from each pack 3 different helminth species were detected. All together, 2 species of helminths were identified in 11.5% of samples, 3 species in 9.6% samples

Table 3. Comparison of mean species richness between wolf packs.

Pack	Number of samples tested	Mean species richness ± SE	Range
Galwica	24	1.91 ± 1.11	0–3
Malga	17	1.45 ± 1.13	0–2
Pogubie	3	1.59 ± 1.33	0–3
Spychowo	2	2.00 ± 1.41	0–3
Ulesie	6	2.33 ± 1.22	0–4

SE - standard error of the mean

Table 5. Comparison of prevalence rates due to two detection methods.

Helminth species	Prevalence (% infected)			Association (Fisher's test)
	Flotation technique	Decantation technique	Total	
Trichuris vulpis	11.4	43.2	38.5	NS
Toxocara canis	9.1	11.4	13.5	***
Uncinaria/Ancylostoma	11.4	31.8	30.8	*

NS - not significant

and in the single sample from Ulesie pack we found ova of 4 different helminth species. The distribution of helminth species did not differ significantly from both positive and negative binomial and Poisson distributions, but showed the lowest fitness to the normal model (χ^2=3.97, df=2, p=0.1).

Prevalence of higher taxa

Helminths. The overall prevalence data are summarized by pack in Table 1. At the highest taxonomic level (all helminths combined), there were no statistically important differences between the packs. A very similar pattern was found for nematodes and cestodes (Tab. 1). However, the pack was on the border of significance as an important factor affecting the prevalence of unidentified tapeworms (χ^2=8.2, df=4, p=0.08). The oncospheres were not found in the Malga and Ulesie packs, but were relatively prevalent in Galwica, Pogubie and Spychowo packs (12.5%; 1 infected per 3 studied samples; 1 infected per 2 studied samples, respectively).

Intestinal protozoa. The pack was an important factor affecting the prevalence of intestinal protozoa (χ^2=16.7, df=4, p=0.002), depending mostly on *C. parvum* distribution. The oocysts were found in all 6 samples from the Ulesie pack (100%) and the prevalence of protozoa decreased gradually in the Malga and Galwica packs, (Tab. 1). No protozoan infective stages were found in 2 samples from Spychowo pack territory.

Prevalence of species

Helminths. The prevalence of the component species (>10% for *T. vulpis*, *Uncinaria*/*Ancylostoma*, *T. canis*) was analyzed by χ^2 statistical test with the pack as a factor. Because of the small number of samples from Pogubie and Spychowo packs there were no statistically significant differences in prevalence of these nematodes between the packs (Tab. 1). Among the other parasite species, *D. caninum* was found only in the Galwica and Ulesie packs; however, because of a very low overall prevalence this observation was not supported by statistics. None of the remaining 2 species showed sufficiently high overall prevalence to facilitate analysis (Tab. 1).

Intestinal protozoa. 51 fecal samples were surveyed for *C. parvum* infections and 33 samples for *Giardia* spp.

Table 4. Abundance (geometric means ± SD) of parasite taxa in studied wolf packs.

Taxon	Species	Number of samples tested	Galwica n=24	Malga n=17	Pogubie n=3	Spychowo n=2	Ulesie n=6
Helminths							
Nematodes	*Trichuris vulpis*	52	1.87 ± 1.21	1.77 ± 1.26	1.26 ± 1.72	0	2.45 ± 1.47
	Uncinaria/Ancylostoma	52	1.70 ± 1.22	1.31 ± 1.26	1.91 ± 1.74	5.39 ± 1.96	2.49 ± 1.48
	Toxocara canis	52	1.36 ± 1.23	1.04 ± 1.27	0	18.44 ± 2.02	1.35 ± 1.50
	Toxascaris leonina	52	1.03 ± 1.05	1.08 ± 1.05	0	0	2.45 ± 1.47
All nematodes		52	3.24 ± 1.33	2.36 ± 1.41	2.00 ± 2.25	19.18 ± 2.71	4.58 ± 1.78
Cestodes	*Dipylidium caninum*	52	1.03 ± 1.04	0	0	0	1.26 ± 1.09
	unidentified tapeworms	52	1.09 ± 1.08	0	1.26 ± 1.23	4.47 ± 1.29	0
All cestodes		52	1.12 ± 1.09	0	1.26 ± 1.27	4.47 ± 1.34	1.26 ± 1.18
All helminths		52	3.49 ± 1.33	2.36 ± 1.40	2.08 ± 2.34	19.67 ± 2.68	4.65 ± 1.77
Intestinal protozoa							
	Cryptosporidium parvum	51	8.55 ± 1.92	85.17 ± 2.1	nd	0	69.61 ± 3.50
	Giardia spp.	33	12.71 ± 3.26	59.92 ± 2.99	nd	nd	166.65 ± 7.75

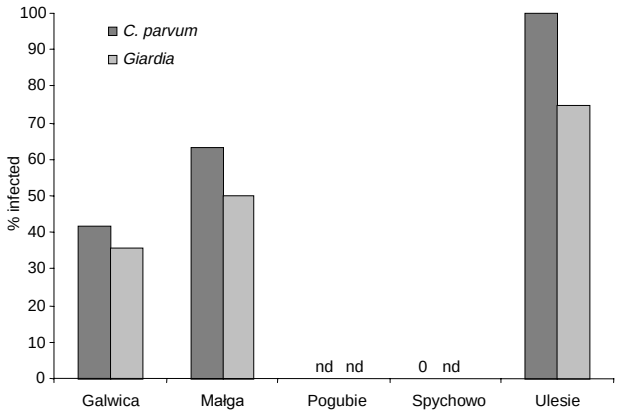

Figure 2. Prevalence of *Cryptosporidium parvum* and *Giardia* spp. in packs.

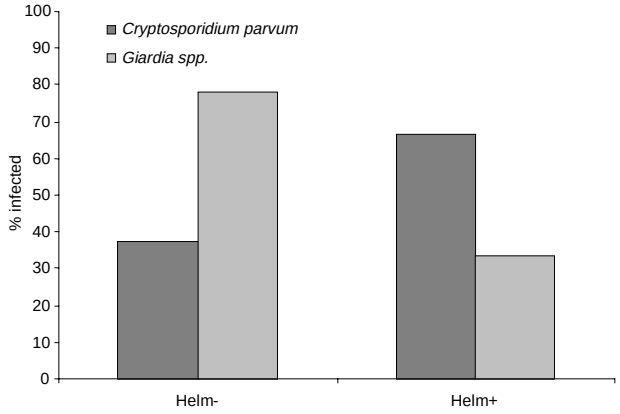

Figure 3. Co-occurence of microparasites and helminths.

Both parasites were found in wolf feces. Almost 55% of samples (28/51) were positive for *C. parvum* oocysts and 45.5% (15/33) for *Giardia* sp. cysts (Tab. 1).

There were significant differences in *C. parvum* distribution between packs (χ^2=13.55, df=3, p=0.004) (Fig. 2). All 6 scats collected in Ulesie pack territory contained oocysts. The prevalence was also high in the Malga pack (63%) and slightly lower in the Galwica pack (42%). Two samples from Spychowo pack were negative.

In the minimum sufficient model for *C. parvum*, the interaction between prevalence of this parasite and prevalence of nematodes was on the border of significance (n=46, χ^2=3.76, df=1, p=0.05). The prevalence of protozoan parasites was higher in samples containing also nematode eggs in comparison to samples without them. Similar but stronger interaction between micro- and macroparasites prevalence was found for *C. parvum* and total helminths (χ^2=6.53, df=1, p=0.01) (Fig. 3a). The goodness of fit for the first minimum sufficient model of *C. parvum* and nematodes was satisfactory (χ^2=17.05, df=21, p=0.71).

The differences in *Giardia* spp. distribution between packs were not significant (Tab. 1) (Fig. 2) and the statistical analysis revealed only 1 negative interaction between *Giardia* spp. prevalence and the prevalence of nematodes (χ^2=7.01, df=1, p=0.008). Opposite to *C. parvum*, *Giardia* spp. prevalence was higher in samples without nematodes eggs. Similar but weaker association was found for the prevalence of *Giardia* spp. and helminths (χ^2=5.19, df=1, p=0.02) (Fig. 3b). However, the goodness of fit of this minimum sufficient model was not very satisfactory (χ^2=22.81, df=17, p=0.16). We found no interactions between prevalence of *Giardia* and *C. parvum*.

Frequency distributions and measures of aggregation

Helminths. Quantitative analysis was confined to the 4 species that showed an overall prevalence >10%. It was not possible to test the distribution of parasites for goodness of fit to the negative binomial distribution because of insufficient degrees of freedom. For *Uncinaria*/*Ancylostoma* and *T. canis* the distribution of parasite ova did not differ significantly from both positive binomial and Poisson distributions, but showed the lowest fitness to the normal model. For *T. vulpis* the fitness to both positive binomial and Poisson distributions was close to the border of rejection (χ^2=2.9, df=1, p=0.09 and χ^2=3.3, df=1, p=0.07, respectively) and the normal model was rejected (χ^2=43.48, df=1, p<0.001). For unidentified tapeworms no distributions could be fitted because of insufficient degrees of freedom arising from too few samples. Otherwise, the 4 species distributions were closer to a negative binomial distribution than to the others, which is reflected by the value of index of dispersion (*T. vulpis I*=13.09; *T. canis I*=277.69; *Unc./Anc. I*=16.69; unident. tapeworms *I*=15.73).

Intestinal protozoa. For *C. parvum* the distribution of oocysts did not differ significantly from both positive an negative binomial and Poisson distributions, but the normal model was rejected (χ^2=9.4, df=1, p=0.002). For *Giardia* spp. the distribution of cysts did not differ significantly from both positive an negative binomial, normal and Poisson distributions. For both parasites index of dispersion was very high (*C. parvum I*=4880.5; *Giardia* spp. *I*=22797.0) indicated highly overdispersed data.

For this reason, statistical analysis of abundance were carried out using log 10(x+1) transformed data, with normal errors and results were expressed as geometric means.

Abundance of infection. The abundance of each species and higher taxa (overall and by pack) is summarized in Tables 1 and 4.

Helminths. There was no significant variation in abundance of *T. vulpis* and *Uncinaria*/*Ancylostoma* between the packs. However, the pack was an important factor affecting the abundance of *T. canis* ($F_{4, 51}$=3.79, p=0.01) (Tab. 4). An approximately 10-fold higher abundance of parasite eggs was found in Spychowo samples (n=2). The pack factor affected also the abundance of unidentified tapeworms ($F_{4, 51}$=8.11, p<0.001) (Tab. 4). Again, a 4 times higher abundance of this parasite was

found in 2 samples from Spychowo territory. None of the remaining parasites were present in sufficient samples to merit analysis.

Intestinal protozoa. The abundance of *C. parvum* differed slightly between packs ($F_{2, 50}$=2.39, p=0.08) (Tab. 4). The abundance was the highest in the Malga pack (geometric mean number of oocysts/ml [GMO]= 85.2 ± 2.1) and high in the Ulesie pack (GMO = 69.6 ± 3.5), but much lower in Galwica samples (GMO = 8.6 ± 1.9). The abundance of *C. parvum* was affected by interaction of pack and nematode prevalence (3-way ANOVA on LOG10 (x+1) transformed oocyst output, with normal errors, $F_{2, 44}$=2.44, 0.05<p<0.1). In the Galwica and Malga packs, the higher oocyst output was found in samples containing also nematode eggs in comparison to samples without them (Galwica: 3.19 versus 3.00; Malga: 12.45 versus 5.51). On the contrary, in Ulesie pack the association was reversed according to 1 sample negative for nematodes, but containing high amount of oocysts.

Because of a lower sample size (n=33) the differences in abundance of *Giardia* spp. between packs were not significant (Tab. 4). However, the abundance was affected by nematodes prevalence (3-way ANOVA on LOG10 (x+1) transformed cyst output, with normal errors, $F_{1, 29}$=4.75, 0.025<p<0.05). Geometric mean number of excreted cysts/ml was much higher in samples free of nematode eggs (403.5 *vs* 11.2).

Comparison of flotation and decantation methods for helminth detection. Forty four fecal samples were analyzed using 2 detection techniques - flotation and decantation. The association between these 2 methods was estimated by Fisher's exact test with Yates correction (using Instat software). The comparison of prevalence estimated accordingly to these 2 methods is given in Table 5. Generally, higher prevalence rates were given by decantation techniques. The strongest association between 2 detection methods was demonstrated for detection of *T. canis* - 96% of compatibility (Fisher's exact test: p=0.003). Weaker association was found for detection of *Uncinaria*/*Ancylostoma* group (75% of compatibility; Fisher's exact test: p=0.03) arising mostly from better detection of L3 larvae by means of decantation technique (Tab. 5). No association was found for *T. vulpis*, primarily because of much higher sensitivity of decantation technique for this parasite detection (Tab. 5). Again, none of the remaining parasites were present in sufficient samples to merit analysis; however *D. caninum* infections (n=2) were detected only due to flotation technique.

DISCUSSION

During the coprological study on 57 wolf samples from NE Poland we have detected at least 7 helminths and 2 protozoan intestinal parasites. The total species richness of studied helminth component community is in the range of published results: from 5 in a Quebec study, through 12 in Spain to 24 in Byelorussia [25, 31, 33]. The overall prevalence of helminths was 63.5% which is relatively low comparing with autopsies data from the other parts of Europe (100% in [15], 96% in [32], 80% in [33]). However, the coprological survey may underestimate the helminth prevalence and even 50-60% of *Taenia* spp. infections may remain undetected [25], thus we can suppose that the real prevalence in the investigated population was much higher than reported. Probably for that reason cestodes seemed not to be very abudant in the studied area, and the majority of detected eggs belonged to nematodes. The mean species richness of infracommunity was 1.0 ± 0.14; twice as low as than in the study of Segovia *et al.* [31], and maximum number of species for infracommunity was 4, similar to 5 reported in a Spanish study. All helminth species identified in our study were identified in wolves in Europe [15, 31, 32, 33] and in North America [10]. In a former study on wolf parasites in Poland [35], the only paper from our country, 5 helminth species were described, including *Trichinella* sp., which was not studied in the current paper. Two of the common species detected by Soltys [35], *Alaria alata* (Trematoda) and *Crenosoma vulpis*, were not recorded in our study. The only species in common with our results was *U. stenocephala*. Such differences were most probably caused by crucial events that took place in the Polish wolf population history since the 1950s. In the 1960s, wolves underwent a heavy population reduction due to intensive persecution. After the collapse in the early 1970s, the population was rebuilt by animals migrating from the east [29]. Thus, it is likely that wolf parasite component community, described in [35], was extinct with host extinction, and the present parasite species richness was established with new hosts from the east. The long list of 24 wolf parasites from Belorussian Polesie [33] supports this hypothesis. However, the other possibility for exchange in parasite species is the transmission from local carnivores (dogs, cats, red foxes), but this needs further studies on the molecular level.

The most frequent helminth species in our study was *T. vulpis* with prevalence of almost 40%. This is much higher than in studies carried out in Italy (9% in [15]), Spain (10–11%, [31, 32]) and Byelorussia (4% in [33]) and this parasite was not recorded previously in Poland [35]. However, this is also a new parasite species in Spanish wolves [32] and in present Polish studies on red foxes the prevalence of *T. vulpis* was also high - 16.1% [6] suggesting possible route of transmission. The prevalence of this parasite is believed to increase with host age because of accumulation of these long-living parasites which are also able to depress host immune response [8, 15]. The second most prevalent nematodes were a group of *U. stenocephala*/*A. caninum* exceeding 31%, similar to other results from Europe. *U. stenocephala* was reported as a core species in Italy and Spain (prevalence >50%), whereas prevalence of *A. caninum* was usually lower, in the range of 8–16% [15, 31, 32, 33]. We recorded a relatively high prevalence of *T. canis* in

the studied area (14%), but there were considerable differences in the prevalence (0–50%) between packs. *T. canis* causes the 'larva migrans visceralis' syndrome in humans, therefore the higher distribution of this parasite in the environment the higher risk of human infection [2]. Due to its life cycle, including transplacental transmission between female and offspring, this parasite is believed to be a parasite of juveniles and there are available environmental data supporting this hypothesis [2, 15]. The distribution of this parasite in European wolves differs from 6% in Spain, 17% in Italy up to 21% in Byelorussia [15, 31, 32, 33]. However, in Poland, a very high prevalence of *T. canis* (40%) was noted in red foxes, and in Slovakia in stray dogs (32%) [2, 6] creating the opportunity for increased transmission to wolf populations.

Taking together *T. vulpis* infections as an indicator of 'advanced' host age and *T. canis* infection as an indicator of 'juvenile' host age we can describe the Galwica, Malga and Pogubie packs as consisting mostly of adults, and the Spychowo pack as territory of juveniles (Tab. 1). The other 2 satellite species - nematode *T. leonina* and cestode *D. caninum* - are also rare species in European wolf populations, ranging from 2–6% in Italy and Spain; and only in Byelorussia exceeding 14% and 15%, respectively [15, 31, 32, 33].

To our knowledge, the present paper is the first record of *Giardia* spp. and *C. parvum* in wolves. To date, numerous studies have reported on *C. parvum* and *Giardia* spp. occurrence in livestock and pets [13], and in some regions of the world more than a half of human cryptosporidiosis cases are caused by the zoonotic *C. parvum* strain [23, 40]. However, little is known about the role wildlife as a reservoir of opportunistic pathogens for humans. Some authors suggest that their contribution in outbreaks of cryptosporidiosis or giardiosis is uncertain or its significance is small [26, 34], while others consider them as an important reservoir of these parasites [3, 5, 38]. The environmental surveys on *C. parvum* and *Giardia* spp. infections in larger mammals are few. In Poland, *C. parvum* has been previously reported in sheep [19], calves [7] and horses [20], and *Giardia* was found in a few mammal species in Poznań zoological garden [21]. The present study contains the first data on intestinal microparasites in wolves and suggests a wide distribution of both species at least in Mazury lake district. Because these parasites are commonly found in young animals, this may suggest a large proportion of young wolves in studied packs.

Wolves themselves were reported to contribute significantly in zoonoses in the southern region of Europe [15, 31, 32]. Segovia and his colleagues [32] showed, that in Spain wolves were involved in zoonoses as major parasite reservoir; however it was suggested that the epidemiological importance of wolves is low compared to foxes [24]. On the other hand, opposite to the other carnivores inhabiting Europe, wolves are very mobile 1 pack may daily cover over 40 km, and when colonizing new areas wolves are able to migrate for even longer distances [18]. Their ability to spread parasite infective stages is connected with their social behavior. Wolves mark their pack territory with scats and urine which are usually deposited in exposed places such as forest roads, forest verges, etc. [42]. Rain and melting snow wash out the parasite infective stages from the feces and eggs, while cysts or oocysts remain on the ground surface or in soil even when the scat had already been decomposed. Consumption of unwashed forest fruits may lead to human infection, and direct accidental contact with wolf feces may results with infections of companion animals (dogs, cats).

Recent studies revealed a marked genetic diversity among different strains of *Cryptosporidium* and *Giardia* [40]. Even for 'zoonotic' parasite species or strains the role of various animals in epidemiology was only partially confirmed and the risk of zoonotic infection cannot be determined without better knowledge on the distribution of the particular genotypes [26]. Further studies are needed for determination of parasite genotypes circulating among wolves.

CONCLUSIONS

In the present study we reported on the wide distribution of helminth and intestinal protozoan infection in wolves in Mazury lake district in the region of NE Poland extensively used by tourists. The identified parasite fauna consists of several micro- and macroparasites of interest for public health. Thus, the increasing population of wolves in Poland should also be treated as growing reservoir for human pathogens.

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