

Potential risk of zoonotic infections in recreational areas visited by *Sus scrofa* and *Vulpes vulpes*. Case study – Wolin Island, Poland

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ABSTRACT. The relation between intestinal parasite prevalence in wild boars and red foxes and the sanitary condition of the soil in recreational estates were determined. The analysis was made based on 36 samples of boar faeces and 22 samples of fox faeces, collected in their habitat as well as 60 samples of soil from two recreational areas. Two methods were used for faecal samples – flotation and direct faecal smear; and flotation in $NaNO_3$ for soil samples examination. Zoonotic nematode eggs were recovered from 25.5% of boar faecal samples; they were *Ascaris suum* (22.2%) and *Trichuris suis* (5.6%). Other parasites found were: *Metastrongylus* sp. (69.4%), *Oesophagostomum* sp., *Strongyloides* sp. (36.6%) and *Physocephalus* sp. (8.6%) as well as coccidia (69.4%). In fox faeces, zoonotic nematode eggs were recovered from 31.8% of samples, and they were *Toxocara canis* (27.2%) and *Ancylostoma caninum* (18.2%). Tapeworm eggs were found in 36.4% of samples including *Taenia* sp. (22.7%). The presence of *Uncinaria stenocephala* (45.5%), *Capillaria* sp. (36.4%), *Trichuris vulpis* (4.5%) and coccidia (40.1%) was also detected. It was shown that both, flotation and faecal smear, as mutually complementary should be used for higher rate of detection of parasites in faeces. No eggs of zoonotic helminths in soil from recreational areas were found despite these areas were accessible to wild animals and pets. This could be explained by characteristics of the soil (loose sand soil) as well as by behaviour of the parasite hosts in the examined areas.

Key words: recreational areas, soil contamination, red fox, wild boar, zoonotic helminths, Poland

Introduction

Wildlife synanthropization is a rapidly developing phenomenon in Europe. Large and medium-size mammalian species most commonly encroaching on urbanized areas are the wild boar *Sus scrofa* and the red fox *Vulpes vulpes* [1–4]. Their number has grown considerably over the past few decades, which can be attributed to overcoming of the fear of humans, expansion of land under development, greater feeding opportunities and in the case of foxes the broad-scale rabies vaccination. [4–8]. Frequent presence of wild animals in populated areas causes psychological problems (e. g., feeling disturbed, fear of attack, or collisions with vehicles), as well as deterioration of the appearance and health safety of housing estates (spilled litter, and ever-present faeces). Moreover, wildlife can be a health hazard to people and pets, as they are host to several of both, zoonotic microorganisms and parasites [4,9–11].

On the Baltic shore, *Sus scrofa* has found excellent living conditions, and even at daytime whole packs haunt the seashore, playgrounds, camp sites and recreational estates (Fig. 1). The presence of red foxes is also common at night, especially in built up areas. The very popular destination is Wolin Island, with its outstanding natural beauty (nearly half of the island belongs to a national park) and mild climate. Throughout the year, it is visited by thousands of tourists and holiday-makers, especially in the spring and summer times. The conditions prevailing there make the place very attractive for boars and foxes, as the recreational estates are surrounded by old forest, in fact they are like islands amidst more or less natural forests. Owing to such favourable trophic conditions, the populations of these two species have been growing. According to the information provided by the Forestry District of Międzyzdroje, wild boar density in the Wolin Island is estimated today at 5.3 specimens per square kilometre and red

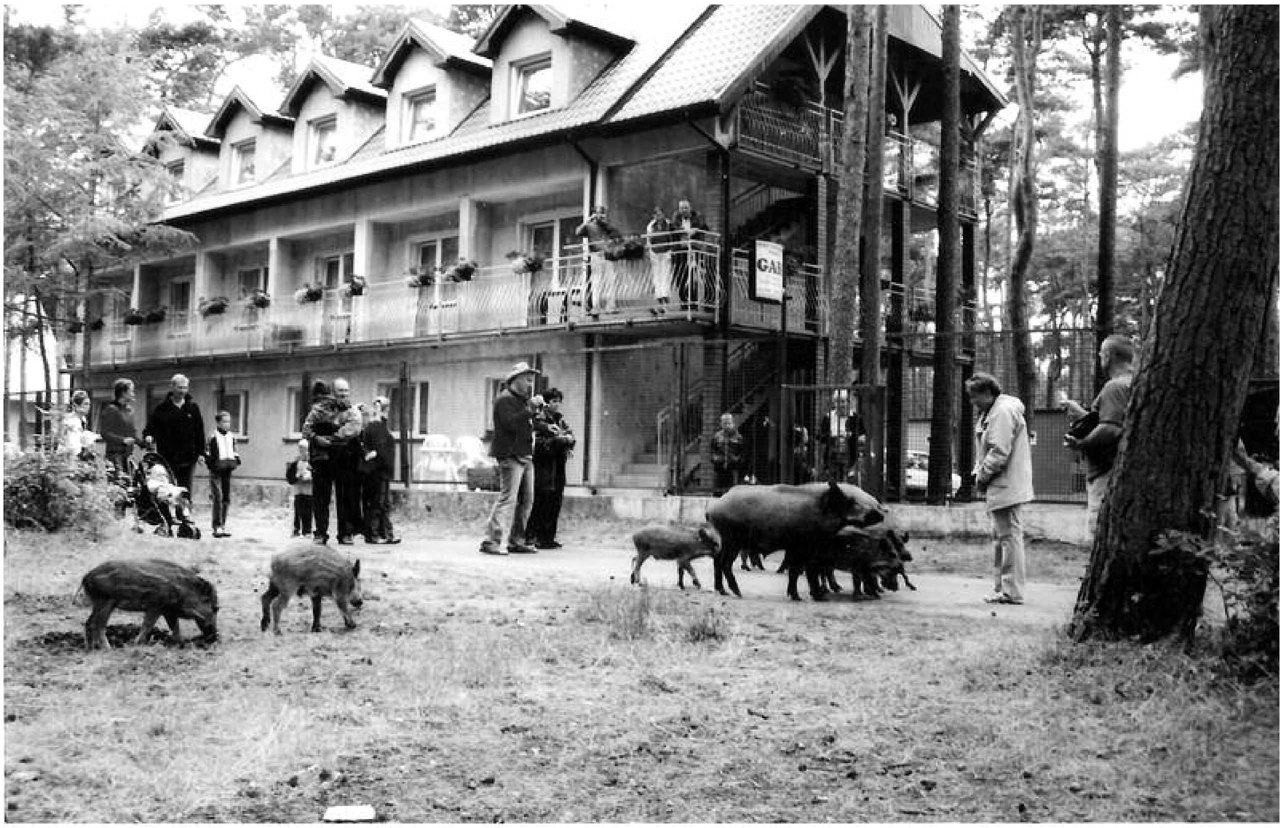

Fig. 1. Wild boars close to the holiday estate examined in Międzywodzie (fot. J. Juszkiewicz)

foxes 1.2 specimens per square kilometre. These characteristics of the island made us to choose the area for the study.

We focused on the relation between the prevalence of intestinal parasite in wild boars and red foxes and the sanitary condition of the soil in the seaside recreational estates. The presence of the parasites in the faecal samples collected in the habitat of the animals was examined first and then the soil samples from the anthropogenic environments were examined. Neither soil contamination nor intestinal parasite prevalence in red foxes and wild boars at Wolin Island have been examined before. Current information on the degree of their invasion elsewhere in Europe is also scant [12–14]. So, this study has provided new information about the parasitic fauna of red foxes and wild boars at the island and applied a novel, broaden approach to the evaluation of public and veterinary health hazards when human habitats are penetrated by free-ranging animals infected with zoonotic parasites.

Material and methods

The study was conducted at the western Polish Wolin Island, situated in the southern part of the Pomorska Gulf (Fig. 2). The island is separated from the mainland by the delta of the River Oder, i.e., the strait formed by the River Dziwna, the Kamień Bay and the Szczecin Bay; while it is the River Świna that separates the Wolin Island from neighbouring Island of Usedom. The Wolin Island is 245 km^2 large, most of it is forested, and nearly half the island represents a protected area within the Woliński National Park established in 1960.

Faeces examination

Sampling of wild boar and red fox faeces was carried out two times. In September 2007, 22 fox samples and 29 boar samples were collected from several sites spread across the island. Then, in July 2009, 7 samples of boar faeces were collected, but this time within 1 kilometre from the recreational estates monitored for soil contamination with geohelminth eggs. Samples (approximately 20 g), were picked up from boar routing areas in the open fields and the forest, and within 100 metres from fox burrows. Foresters of the Międzyzdroje Forestry District had good knowledge of the area and helped to locate and identify wild animal faeces. Material was immersed upon collection in a 4% formalin solution to prevent fungi and bacteria growth, and then transported to the laboratory. From each sample, four portions of 2 g each, were taken to the analyses. They were examined by Sheather's flotation (2 portions) and by direct faecal smear (2 portions). The specimens were examined using a light microscope under 10×40 magnification. Parasite egg and cyst identification was done on the basis of their size and shape as well as on the basis of egg-shell transparency and thickness. The measurements were taken with the aid of a microscope with a computer software (Motic Images Plus).

Soil examination

Soil samples were collected at recreational estates of Międzywodzie and Grodno, both well maintained and having well developed recreational infrastructure and facilities. These two areas were potentially exposed to biological contamination related to defecation of synanthropic animals and pets. Boars and foxes were often observed within or in the vicinity of these premises while dogs are allowed in both establishments (Fig. 1). A comparison of the two estates is presented in Table 1. In July 2009, sixty 250 ml soil samples were collected (30 from each estate), including 6 samples from sandboxes used by children (2 samples from Międzywodzie and 4 from Grodno). The material was examined in the laboratory by modified Dada flotation technique [15].

Results

Faeces examination

Of 36 samples of boar faeces, parasites were re-

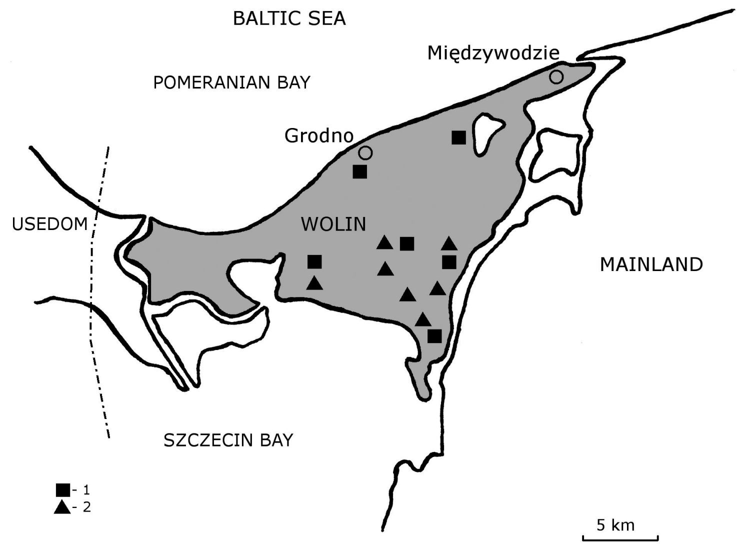

Fig. 2. Distribution of faeces with parasites eggs/oocyst of wild animals on Wolin Island. 1–*Sus scrofa*, 2–*Vulpes vulpes*.

covered from 30 samples (83.3%). Zoonotic nematode eggs were present in 9 samples (25.5%), from eight of which (22.2%) 17 *Ascaris suum*, eggs were recovered, and in two samples (5.6%) 3 *Trichuris suis* eggs were identified. Lungworm eggs *Metastrongylus* sp., were frequently found in wild boar faeces – a total of 170 were recovered from 25 samples (69.4%). Coccidia were just as frequent – found in 25 samples (69.4%), while the total number of oocysts recovered was 160. Nematode eggs of

Table 1. Description of holiday estates studied for soil contamination with helminth eggs. Wolin Island, 2009.

	Grodno	Międzywodzie
Total area (recreational facilities)	1600 m^2 (400 m^2)	6000 m^2 (1000 m^2)
Site description	thick, mixed forest, far from urbanized areas (within the borders of national park)	pine forest, urbanized area
Distance from the Baltic shore	100 m	200 m
Soil structure	loose sand soil	loose sand soil
Shading	high throughout the day	moderate
Sports and recreational facilities	very good (sports grounds, children's playgrounds, a sandbox)	very good (barbecue area, children's playgrounds, a sandbox)
Fencing	total, with crossings for large animals	total
Accessibility to animals	foxes and boars seen on the premises at night	foxes seen on the premises at night, boars approaching the fences during the day
Dogs	allowed	allowed
Number of samples	30	30

Table 2. Results of examination of faecal samples of wild boars (*Sus scrofa*) in Wolin Island (2007, 2009)

	As	Ts	M	S	O	P	B	Cc
Flotation								
Positive (*n*)	2	2	19	7	8	2	1	15
Prevalence (%)	5.6	5.6	52.8	19.4	22.2	5.6	2.8	41.7
Range (mean)	1–7 (4)	1 (1)	1–40 (7.1)	3–11 (4)	1–3 (1.7)	1 (1)	1 (1)	1–14 (4.7)
S.D.	4.24	–	9.25	3.11	0.71	–	–	4.48
Smear								
Positive (*n*)	8	1	18	10	10	3	0	23
Prevalence (%)	22.2	2.8	50	27.8	27.8	8.3	–	63.9
Range (M)	1–2 (1.1)	1 (1)	1–6 (2)	1–9 (2.6)	1–3 (1.5)	1–5 (3,8)	–	1–13 (3.9
S.D.	0.35	–	1.51	2.50	0.82	1.89	–	3.06
Flotation and smear								
Positive (*n*)	8	2	25	13	13	3	1	25
Prevalence (%)	22.2	5.6	69.4	36.1	36.1	8.3	2.8	69.4

Explanations: As–*Ascaris suum*; Ts–*Trichuris suis*; M–*Metastrongylus* sp.; S–*Strongyloides* sp.; O–*Oesophagostomum* sp.; P–*Physocephalus* sp.; B–*Balantidium* sp.; Cc–Coccidia

Oesophgostomum sp. and *Strongyloides* sp. were found in 13 samples (36.1%), the number of eggs was 32 and 61, respectively. *Physocephalus* sp., eggs were less common – 14 eggs were present in 3 samples (8.6%) (Fig. 2, Table 2). Interestingly, the eggs of this nematode were only recovered from the July 2009 samples. Also, a single *Balantidium* sp., cyst and one unidentified nematode egg were found. All the parasitic species recovered in 2007 from boar faecal samples were also found in 2009 boar faecal samples.

Of the 22 fox faecal samples, eggs of parasites were found in 17 samples (77.3%). Zoonotic nematode eggs were recovered from 7 samples (31.8%), with a total of 15 *Toxocara canis* eggs found in 6 faecal specimens (27.2%) and 15 *Ancylostoma cani-*

Table 3. Results of examination of faecal samples of foxes (*Vulpes vulpes*) in Wolin Island (2007)

	Tc	C	Tv	Us	Ac	Cs	Cc
Flotation							
Positive (*n*)	5	8	1	6	2	7	7
Prevalence (%)	22.7	36.4	4.5	27.2	9.1	31.8	31.8
Range (M)	1–3 (2)	1–9 (3.4)	3 (3)	1–5 (2)	2–3 (2.5)	1–11 (4.1)	1–3 (1.6)
S.D.	1	3.2	–	1.55	0.71	2.93	0.98
Smear							
Positive (*n*)	2	0	0	7	3	4	2
Prevalence (%)	9.1	–	–	31.8	13.6	18.2	9.1
Range (M)	1–4 (2.5)	–	–	1–4 (1.7)	2–5 (3.3)	1–3 (2.5)	2–3 (2.5)
S.D.	2.12	–	–	1.25	1.53	1.00	0.71
Flotation and smear							
Positive (*n*)	6	8	1	10	4	8	9
Prevalence (%)	27.2	36.4	4.5	45.5	18.2	36.4	40.1

Explanations: Tc–*Toxocara canis*; C–*Capillaria* sp.; Tv–*Trichuris vulpis*; Us–*Uncinaria stenocephala*; Ac–*Ancylostoma caninum*; Cs–Cestoda; Cc–Coccidia *num* eggs in 4 samples (18.2%). Other parasite eggs found in foxes included nematode *Uncinaria stenocephala* eggs in 45.5% of samples (19 eggs recovered from 10 samples), *Capillaria* sp. in 36.4% (27 eggs in 8 samples) and *Trichuris vulpis* in 4.5% of samples (3 eggs recovered from 1 sample). Tapeworm eggs were found in 8 samples (36.4%), with *Taenia* sp. recovered from 5 samples and *Diphylidium caninum* from 6 samples (27.2%). Nine samples (40.1%) contained 15 coccidian oocysts. Furthermore, in one fox faecal sample, a single *Ascaris* sp. and *Metastrongylus* sp. eggs were found together with *U. stenocephala* egg (Fig. 2, Table 3). In 18% of samples (boars and foxes) some other parasites were identified, such as *Syphacia* sp., *Trichosomides* sp. and mite eggs (Acarina).

The two methods of recovery of parasites from faeces – flotation and direct faecal smear – have proved to be mutually complementary. Faecal smear was a more effective technique with wild boar faeces – 213 eggs or cysts from 29 faecal samples were recovered using this method. Flotation, on the other hand, identified 110 eggs or cysts in 26 samples. With the fox faeces, situation was different. More parasites were found by flotation method, namely 91 eggs or cysts in 17 samples, while faecal smear was accounted for 39 eggs or cysts recovered from 10 samples. In general, when it comes to identification of nematode eggs and protozoan cysts from both hosts – the boar and the fox – faecal smear proved more effective. For nematode eggs, the difference in the effectiveness of these methods used (as referred to the prevalence) was statistically significant ($p<0.05$) (Fig. 3). It has to be emphasized, however, that in the case of both hosts (foxes and boars) there were several cases of parasite recovery by one method only, i. e., either flotation or direct faecal smear).

Soil examination

None of 60 sand soil samples from the two seaside recreational estates were positive for eggs of zoonotic helminths. Only in one sample from Grodno a single *Metastronglus* sp. egg was found and in one sample from Międzywodzie a single egg of *Capillaria* sp. was found.

Discussion

The Baltic shore is an area where wild boars and red foxes strongly encroach on human environment.

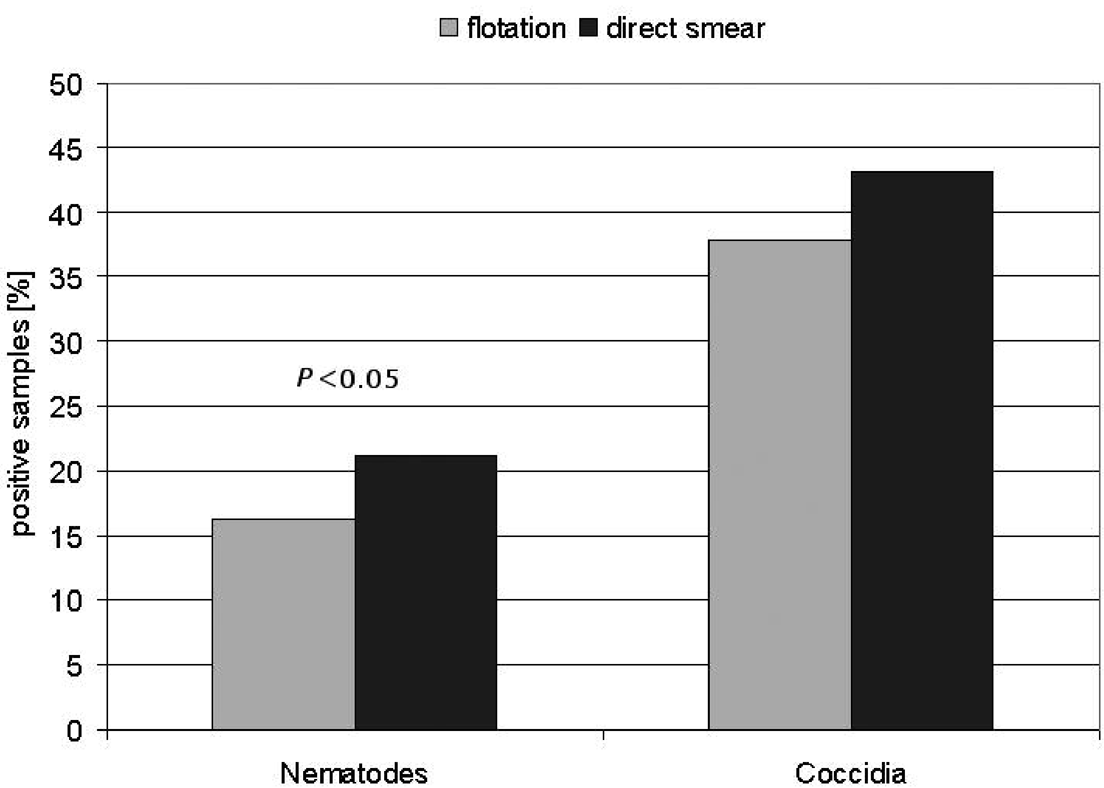

Fig.3. A comparision of efficiency of two methods used for isolation of parasites eggs and oocysts from faeces of wild boars (*Sus scrofa*) and foxes (*Vulpes vulpes*)

Table 4. The prevalence of intestinal parasites in wild boars (*Sus scrofa*) in various parts of Europe

	Samples N	Prevalence (%)							References
		As	Ts	M	S	O	P	Cc	
Kampinos National Park, Poland	37	28–60	52–82	80–100	–	19–60	36–60	–	[16]
Spain	47	2	–	85	–	–	6	–	[13]
France Spain	9 15	44.4 0.0	33.3 0.0	66.7 46.7	22.2 28.6	22.2 0.0	22.2 35.7	–	[12]
West Pomerania, Poland	147	29.3	8.2	12.9	–	54.4	–	58.5	[17]
Corsica	160	0–30	–	10–35	–	–	–	–	[18]
Slovak Republic	411	6.1	–	–	–	–	–	–	[19]
Saaremaa Island, Estonia	100	9	21	82	–	–	–	–	[20]

Explanations: As–*Ascaris suum*; Ts–*Trichuris suis*; M–*Metastrongylus* sp.; S–*Strongyloides* sp.; O–*Oesophagostomum* sp.; P–*Physocephalus* sp.; Cc–Coccidia

Coprological examinations show that both animal species at Wolin Island are frequent hosts for zoonotic parasites. Twenty-seven percent of foxes were infected with *T. canis* and 18% of samples contained *A. caninum*. *Taenia* sp. was also frequent (27.2% positive samples), among which *Echinococcus* sp. eggs cannot be ruled out. In wild boars, the prevalence rate of *A. suum* was 27%, and of *T. suis* – 6%. The results obtained by the coprological examination methods have been basically consistent with results obtained in recent years by other authors who utilized animal necropsy (Tables 4,5),

Table 5. The prevalence of intestinal parasites in red foxes (*Vulpes vulpes*) in various parts of Europe (since 2000)

	Samples *N*	Prevalence (%)							References
		Tc	Tl	Tv	Us	Ac	Cs	Cc	
Guadalajara, Spain	67	39.8	52.2	38.8	58.2	–	1.5–4.4	–	[21]
Poznań region, Poland	92	16.3	–	–	–	–	–	–	[22]
Western Poland	1909	25.5	0.9	16.1	26.0	11.7	1.0–63.8	–	[23]
Belarus	?	25.5	18.1	22.3	40.4%	3.2	3.2–27.7	–	[24]
Great Britain	588	61.6	0.3	0.3	41.3	–	0–20.7	–	[11]
Slovak Republic	310	8.1	47.1	–	–	–	–	–	[25]
West Pomerania, Poland	165	34	1	10	26	7	–	–	[17]
Białowieża Primeval Forest, Poland	22	13.6	–	27.3	27.3	–	4.5	4.5	[26]
Denmark	1040	59.4	0.6	0.5	68.6	0.6	0.3–35.6	–	[7]
Brussels, Belgium	160	17.9	–	–	–	–-	0 *Echinococcus*	–	[10]
Geneva, Switzerland	228	44.3	8.0 – 59.6	–	78.2–20.0	–	54.3	–	[1]

Explanations: Tc–*Toxocara canis*; Tl–*Toxascaris leonina*; Tv–*Trichuris vulpis*; Us–*Uncinaria stenocephala*; Ac–*Ancylostoma caninum*; Cs–Cestoda; Cc–Coccidia and have been similar to the results from the Polish province of Zachodniopomorskie [17]. These similarities are not surprising in view of the fact that the Wolin Island lies within this province and due to proximity to mainland, the island is not really completely isolated. It was observed by island's inhabitants that boars swam across the Dziwna River.

The indirect method for determination of parasite prevalence in wild animals, through faecal sample collection in the animals' habitats, has proved to be effective. It was possible to relatively quickly assess present situation in terms of intestinal infection of wild animals without euthanizing them. Another advantage is the fact that it can be used without restriction in protected areas (e. g., national parks). It should also be emphasized that when microscopic examination is supplemented with other diagnostic techniques (e. g., immunological or molecular), it can provide us with even more information than necropsy (i. e., species identification in tapeworms, protozoans or even microorganisms) [4,11]. However, the method has its weaknesses too; it is time-consuming because of the need to find animal defecation sites and it requires the experience in identification of faeces of specific wild animals species. Besides, heavy rains wash down the faeces quickly; faeces are also decomposed by invertebrates (e. g., *Geotrupes stercorarius* and *Arion rufus*), which makes them more difficult to find and analyze. These obstacles, however, are minor in comparison to the advantages mentioned above.

After comparison of results obtained with these two techniques for egg recovery from animal faeces (i. e., faecal smear and flotation) we conclude that both methods should be used concurrently for the sake of result accuracy, and preferably should be conducted in duplicates as in other studies [27]. These methods have proved to be complementary. And although faecal smear reveal more eggs in a larger number of samples (Fig. 3), its effectiveness was neither more efficacious for both host faeces examination, nor always supreme for identification of parasite species or genus.

During fox faeces examination, we identified single eggs of non-specific parasites, namely one egg of *A. suum* and one *Metastrongylus* sp. As they were recovered from the same faecal sample as the host-specific *Uncinaria* sp., this can be attributed to the fox having eaten the faeces or intestines of an infected wild boar (not uncommon among predators), or to the entry of the eggs into the fox's alimentary tract via ingestion of contaminated soil or vegetation.

No faeces of the hosts were found on the grounds within the recreational estates under examination and no geohelminth eggs were detected there. The results of our parasitological studies and results obtained by other authors investigating the same province [17,28] indicate that the level of boar and probably fox infection has not changed much in recent years. So, the holiday destinations under our study are not threatened with zoonotic parasites, despite the high risk of contamination. Foxes were frequent in these places at night, dogs were allowed at the recreational estates and in Grodno, situated deeply in the forest within the boundaries of the national park, and boars had easy access to the recreational grounds. Although the estate in Międzywodzie was not accessible to boars, they would approach the fence in large packs during the day and bask there, showing no fear of humans (Fig. 1). These results shed new light on the issue of zoonotic infection risk factors. It was generally believed there is a strong correlation between intestinal parasite prevalence in animals and soil contamination in their environment and host exposure to infection [21,29]. However, no such correlation has been observed in our studies. Most probably the reason is its soil structure. Even in areas frequently visited by infected animals the soil was not contaminated with parasites in the upper layers accessible to hosts. The loose sand soil at the examined area does not allow eggs to remain at the surface layers. This was previously proved in experimental studies of *A. suum* eggs which under such conditions revealed very low survivability and relatively quickly leached to lower soil layers [30]. As a result, they were not detected during routine procedures of soil examination, when samples were taken from approximately 5 centimetres deep ground. For the same reason, the eggs become inaccessible to hosts. It is worth mentioning that our earlier observations made in semi-natural conditions over the course of our study were confirmed in the natural habitats. Loose sand soil, due to its high permeability, does not favour the spread of intestinal parasites.

Apart from the soil structure and its characteristics, behaviour of wild boars and foxes could influence the results of soil examination. In the area of recreational estates no faeces of these hosts were observed: feaces were found in boar routing grounds and in the territory of foxes near their burrows. Most likely they defecate reluctantly in anthropogenic environment. Parasite prevalence in boars and foxes that has been stable for years in the examined area indicates the continuous presence of infective stages of parasites in areas not visited by people, where parasites have optimal conditions for survival and transmission among abundantly present hosts.

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